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CHARACTERISTICS OF STELLATE INCLUSIONS IN GIANT CELLS AND THE ASSOCIATED TISSUE REACTIONS *

JOSEPH A. CUNNINGHAM, M.D.

(From the Department of Pathology, Medical College of Alabama, the Lloyd Noland Hospital, and the Carraway Methodist Hospital, Birmingham, Ala.)

This paper reports the study of 31 cases in the tissues of which stellate inclusions in giant cells were demonstrated. Both surgical and necropsy material was included. These cases are listed in Tables I and II.

Goldmann [1] described stellate inclusions in giant cells in 1890. In 1911, Wolbach,[2] in a report of 5 cases, attempted to clarify the nature of these structures and noted their staining reactions in detail. These inclusions—also known as spiculated or asteroid bodies—have been variously thought to be fibrin derivatives (Wolbach), fat crystals (Goldmann), undeveloped spores and filaments of a mold (Ribbert [3]), hypertrophied centrosomes (De Buck and Broeckaert [4]), elastic tissue (Vogel [5] and Letulle [6]), astrospheres (Iwanzoff [7]), and protein derivatives around a lipin core (Herxheimer and Roth [8]). Hirsch,[9] in 1935, reported 36 cases in which he found stellate inclusions in giant cells. He produced "rosette" bodies resembling stellate inclusions by the intravenous injection of a lipin extracted from human omentum, to which he had added cholesterol, stearin, and palmitin. He concluded that "radial inclusions of giant cells observed in tubercle-like granulation tissues are crystalline forms of fats solid at body temperature, such as palmitin or stearin, separated from an oil system containing cholesterol or substances with the physical properties of cholesterol." Friedman,[10] in 1944, reported a case of sarcoid of the spleen in which he found stellate inclusions and, in reviewing the literature, pointed out the frequent finding of these bodies in sarcoid lesions. He concluded that "the asteroid *may* be nonspecific but is characteristically found in sarcoid lesions."

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In view of the renewed interest in sarcoidosis, the present material has been studied (1) to delineate further the histochemical and physical characteristics of stellate inclusions and (2) to categorize the types of associated tissue reactions.

Histochemical and Physical Characteristics of Stellate Inclusions

In this material, stellate inclusions were found only in giant cells. Some of these giant cells contained sudanophilic material in the cytoplasm around the inclusion; others did not. The size of the bodies varied between 10 and 25 μ. They were readily seen in reduced light, in either unstained paraffin or frozen sections. They did not dissolve in 10 per cent potassium hydroxide and resisted 10 per cent formic and hydrochloric acid almost as well as the cytoplasm of the parent giant cell. These inclusions were not soluble in water, ether, chloroform, alcohol, benzene, xylene, acetone, or gasoline. They were isotropic in polarized light.

When stained by the hematoxylin and eosin method after formaldehyde fixation, they were red or pink. After primary fixation in Zenker's fluid and phosphotungstic acid hematoxylin staining, the spines were dark blue or purple and the central core orange-brown, although in many instances this orange core was absent. After other fixatives, the phosphotungstic acid hematoxylin stain gave variable results. Stains for lipids and fatty acid were uniformly negative. Among the stains used were Sudan III, scarlet red, Nile blue sulfate, Fischler's stain for fatty acid, Sudan black, osmic acid, and the plasmal reaction. When tested for carbohydrate, using the periodic acid-Schiff's reagent method, the inclusions remained colorless. The von Kossa test for calcium gave negative results. The Berlin blue reaction for ferric iron failed to stain the inclusion. In tests for collagen, Masson's trichrome stain and Mallory's connective tissue stain showed negative results. Wilder's stain for reticulum was uniformly negative. Tests for elastic tissue were not consistent. Using orcein, very little color was found in the inclusion and the reaction was considered doubtful. Using Weigert's elastic tissue stain, some stellate inclusions took a black stain, but the result was inconstant. (Other investigators [5,8,10] have reported negative results when using Weigert's method and positive findings when safranine and fuchsin were used.) The Fuelgen test for desoxyribosenucleic acid yielded a negative result. The Ziehl-Neelsen method did not stain the inclusions.

In order to test the resistance of the stellate inclusions to heat, sections from 2 cases (one fixed in formaldehyde and stained by the van

Gieson elastic tissue method, the other fixed in Zenker's solution and stained with phosphotungstic acid hematoxylin) were photographed under high power and then placed in a muffle furnace, using a thermocouple to control the heat. The temperature was raised to 250° C., requiring 25 minutes. After cooling, they were re-examined under direct illumination and photographed. The same sections were again placed in the furnace and this time the temperature was brought to 350° C. The time required to do this was 30 minutes, after which they were again observed under direct illumination and photographed. At 250° C. the stellate inclusions showed little change, either by direct observation or by comparison of photographs. At 350° C. there was some distortion, but the stellate form was readily recognizable and well preserved. Also, unstained paraffin sections were brought down to water and the inclusions visualized and marked. The sections were then placed in the muffle furnace and the temperature raised to 650° C. in increments of approximately 70° every 5 minutes. After cooling, a coverslip was applied and sealed around the edges with paraffin. The sections were then examined in direct light and by dark-field illumination. The giant cell outlines were recognized readily, particularly in the dark field, but the stellate inclusions had disappeared completely.

Analysis of Tissue Reactions

The tissue reactions associated with these inclusions are inflammatory and protean. However, if allowance is made for minor variations and overlapping, it is possible to classify them into three main types: (1) foreign body reactions, (2) tuberculoid granulomatous inflammation, and (3) acute and chronic inflammation and repair. Each group is discussed separately.

Foreign Body Reactions

The foreign body reaction is well recognized and amply described in textbooks of pathology. It is characterized by multinucleated giant cells surrounding or engulfing foreign or ectopic material. There is an associated acute or chronic inflammatory reaction, depending on the age of the lesion: Of the 31 cases listed in Tables I and II, 18 showed reactions of this type, and in each instance foreign or ectopic material was demonstrated. The "foreign bodies" included catgut sutures (cases 4, 14, 26, and 29), sodium urate crystals (case 17), ectopic hair, sebaceous material, and/or keratinized débris (cases 19 and 20), degenerated Blastomyces (case 11), and an unidentified foreign body (case 24). In 6 cases, an old area of hemorrhage with tissue necrosis, either with or without the deposition of cholesterol crystals, acted as

TABLE I

Necropsy Material with Stellate Inclusions in Giant Cells

Case no.	Age Sex Race	Anatomical diagnosis	Site	Type of tissue reaction
1	50 F W	Tetanus	Lungs	Perivascular and peribronchial fibrosis with lymphocytic and plasma cell infiltration; intermingled vacuolated macrophages and multinucleated giant cells
2	63 M W	Squamous cell carcinoma, grade 3, left arm (shoulder girdle amputation); metastasis to lymph nodes, lungs, jejunum, and periosteum; lobular pneumonia	Mediastinal lymph nodes	Hemosiderin-laden macrophages in peripheral sinuses; occasional foci of necrosis in the medulla, with scattered vacuolated multinucleated giant cells
3	53 M W	Carcinoma of bladder with extension to prostate and metastasis to pelvic lymph nodes	Tracheal lymph node	Small hyalinized scar in the periphery of the node; multinucleated giant cells in adjacent lympho-reticular tissue
4	65 F W	Periarteritis nodosa	Mesentery (site of previous biopsy)	Catgut suture material surrounded by scar tissue in which there are lymphocytes and multinucleated giant cells
5	36 M W	Sarcoidosis involving heart, liver, lungs, lymph nodes; clinical heart block with Stokes-Adams syndrome	Heart, liver, lung	Tubercle-like collections of epithelioid cells with multinucleated giant cells; older lesions showing intense hyaline scarring and giant cells; (stains for acid-fast organisms negative)
6	41 M W	Glioblastoma multiforme; acute bronchitis; lobular pneumonia	Peribronchial lymph node	Small peripheral hyaline scar with multinucleated giant cells in adjacent lympho-reticular tissue
7	53 M W	Pontine hemorrhage, massive; lobular pneumonia; healed gastrojejunostomy and entero-enterostomy	Peripancreatic and perigastric lymph nodes	Medullary and peripheral sinuses filled with lymphocytes, plasma cells, and eosinophilic polymorphonuclears; scattered multinucleated giant cells with vacuolated cytoplasm in the medulla; (Sudan IV and Scharlach R stains positive)
8	58 F C	Leiomyosarcoma of uterus with local extension and metastasis to lungs and liver	Heart	Small area of subepicardial hyaline scar tissue replacement of heart muscle; in this area, a few lymphocytes and numerous multinucleated giant cells
9	77 M W	Coronary occlusion; pulmonary edema and lobular pneumonia	Mediastinal lymph node	Tubercle-like collections of epithelioid cells without caseation and with scattered multinucleated giant cells; (stains for acid-fast organisms negative)
10	44 M W	Acute alcoholism; fat embolism(?)	Lymph node in gastro-hepatic ligament	Large vacuoles scattered throughout lymph node; collections of vacuolated macrophages, eosinophilic polymorphonuclears and multinucleated giant cells around the vacuoles
11	80 M W	Lobular pneumonia (gram-positive cocci); focal blastomycotic nodules with fibrosis	Lung	Old hyalinized scar with lymphocytic infiltration and multinucleated giant cells with ingested degenerated Blastomyces
12	66 F W	Tuberculosis involving bladder, ureters, kidneys, spleen, and liver	Liver	Periportal spaces with numerous lymphocytes and plasma cells, slight increase in connective tissue, scattered multinucleated giant cells; (stains for acid-fast organisms positive)

the foreign body (cases 2, 18, 25, 27, 28, and 31). In the 3 remaining cases (7, 10, and 15), ectopic fat was found in association with reactions of this type.

Tuberculoid Granulomatous Inflammation

Tuberculoid granulomatous inflammation is characterized by the presence of discrete tubercle-like structures made up of radially arranged epithelioid cells. In some instances typical caseation is seen; in others there is a peculiar type of fibrinoid necrosis. Multinucleated giant cells with varying numbers of nuclei are seen, situated either centrally or peripherally in the granuloma. Older lesions show varying degrees of hyalinization. There were 8 examples of tissue reaction of this type in the 31 cases studied. Four of these were diagnosed as sarcoidosis on the basis of the histologic findings and supporting clinical evidence. Two (cases 9 and 21) showed tubercle-like structures without central necrosis, but these represented isolated findings without clinical confirmation. In the second of these (case 21) there was at least reasonable suspicion that some impregnated foreign material, no longer demonstrable, was the cause of the tissue reaction. The seventh example (case 30) showed an outer zone with epithelioid proliferation, numerous multinucleated giant cells with stellate inclusions, and an extensive zone of central necrosis. Despite careful clinical and histologic study, no etiologic agent could be proved. Finally, case 12 was an established case of tuberculosis in which stellate inclusions were found in several giant cells.

Acute and Chronic Inflammation and Repair

Acute and chronic inflammation and repair comprise a tissue reaction which is harder to define and is more variable than the two preceding types. It is characterized by a cellular infiltration of polymorphonuclear leukocytes, lymphocytes, plasma cells, and monocytes in varying proportions. The giant cells are similar to those in the other two types but are usually less numerous. In some of the older lesions the inflammatory cell response is markedly diminished and the picture is essentially that of a hyaline scar with a few peripherally situated giant cells with inclusions. Five examples (cases 1, 3, 6, 8, and 23) of this type of reaction are included in this group of 31 cases.

Discussion

Studies of the physical and chemical characteristics of stellate inclusions have given further information. The staining reactions indicate that stellate inclusions do not contain stainable carbohydrate, des-

TABLE II

Surgical Material with Stellate Inclusions in Giant Cells

Case no.	Age Sex Race	Diagnosis	Site	Type of tissue reaction
13	48 F W	Sarcoid	Skin, forehead	Tuberculoid granulomatous inflammation without caseation
14	32 F W	Postoperative adhesions	Old operative site in pelvis	Foreign body giant cell reaction around catgut sutures
15	? F W	Carcinoma of breast without axillary metastasis	Axillary lymph node	Beneath peripheral sinuses, large vacuoles collared by epithelioid cells and multinucleated giant cells
16	39 F C	Sarcoidosis	Cervical lymph node	Tuberculoid granulomatous inflammation without caseation
17	52 M W	Gout	Triceps tendon	Foreign body reaction to sodium urate crystals
18	26 M W	Interposition of muscle fragments	Soft tissue between fractured ends of tibia	Fragments of degenerated striated muscle, areas of old hemorrhage and repair with chronic inflammation and numerous scattered multinucleated giant cells
19	? F W	Dermoid	Ovary	Foreign body giant cell reaction around ectopic hair and sebaceous material
20	? F W	Dermoid	Ovary	Same as above
21	33 M C	Sarcoid	Postauricular lymph node	Tuberculoid granulomatous inflammation without caseation

22	27 F W	Sarcoid(?)	Skin, forehead (scar from old injury)	Tuberculoid granulomatous inflammation without caseation; laminated and partially calcified bodies also found in giant cells
23	60 F W	Bronchiectasis	Lung	Peribronchial fibrosis, lymphocytic and plasma cell infiltration, scattered giant cells with vacuolated cytoplasm
24	40 F C	Foreign body granuloma	Lip (history of injury with dental drill)	Heavy plasma cell and lymphocyte infiltration of subepithelial connective tissue, with scattered multinucleated giant cells and ingested unidentified foreign material
25	49 M C	Synovial fibrosarcoma	Periarticular connective tissue, left foot	Old hemorrhage with hemosiderin- and fat-laden macrophages, fibrous repair, and scattered multinucleated giant cells
26	36 F W	Retained suture and foreign body giant cell reaction	Fallopian tube	Acute and chronic inflammation and foreign body giant cells around old catgut suture
27	28 F W	Simple adenoma with old hemorrhage	Thyroid	Numerous hemosiderin-laden macrophages, cholesterol clefts, foreign body giant cells
28	? F W	Endometriosis	Ovary	Old hemorrhage with cholesterol clefts, and foreign body giant cells around these clefts
29	34 F W	Uterus with old operative surface adhesions	Uterus	Surface adhesions with catgut suture surrounded and partially engulfed by foreign body giant cells, scattered lymphocytes and plasma cells
30	29 M W	Tuberculosis(?)	Inguinal lymph node	Focal areas of caseation necrosis, peripheral epithelioid cells and giant cells; (stains for acid-fast organisms negative)
31	64 F W	Intraductal papillomas with old hemorrhage	Breast	Giant cells around cholesterol clefts; scattered macrophages with ingested hemosiderin and fat

oxyribosenucleic acid, fat, ferric iron, or calcium. They are best visualized by using the phosphotungstic acid hematoxylin staining method, as other investigators have shown. They are readily seen in routine hematoxylin and eosin sections. Hirsch [9] is of the opinion that the stellate inclusions represent an altered lipid. The inability to demonstrate stainable lipid suggests that certain chemical changes in the composition of the crystals take place in the tissue so that they become insoluble in fat solvents, and that further changes or additions produce the elastin-like staining qualities. The experiment undertaken to test the resistance of these inclusions to heat indicates that they resist temperatures up to 350° C. with little change. The melting points of the two lipids suggested by Hirsch—stearin and palmitin—are between 65° and 70° C., and therefore it would appear that these bodies are not simple stearin or palmitin crystals. The addition of protein and the formation of a complex lipoprotein is possible. The melting point of this theoretic lipoprotein is not known; consequently it is not possible, on the basis of this new information, to deny such a chemical composition. It seems highly improbable, however, that heat of 350° C. would not materially affect the structure and crystalline form of such a compound.

Micro-incineration at 650° C. results in the complete decomposition of the inclusions. This indicates the organic nature of the structures. If inorganic substances are present, the amounts must be minute. The staining reactions, resistance to heat, and resistance to weak acids and alkalis suggest that these inclusions are made up essentially of protein.

The present study of the tissue reactions associated with giant cells containing stellate inclusions has shown that the most frequent type is the foreign body reaction. Eighteen (58 per cent) of the 31 cases studied are classified under this heading. The types of "foreign bodies" encountered are diverse, with no obvious common physical or chemical characteristics except their ability to produce this type of response.

It was thought worth while to review the literature to check the validity of the present division of the tissue reactions into three types. There are 75 cases reported in which these inclusions are found in giant cells. Of these, 62 are described adequately enough to allow grouping according to the tissue reaction. Thirty-two cases are examples of foreign body reaction and are listed in Table III. The frequency of this type of reaction, with the great diversity of foreign bodies, is strong evidence that these inclusions are not specific for any disease entity.

The next most frequently encountered type of reaction was that listed as tuberculoid granulomatous inflammation. This designation was selected primarily because it is descriptive and does not imply a single etiologic agent. There were 8 examples of response of this type in my material. In only one (case 12) was it possible to demonstrate

TABLE III

Thirty-Two Cases from the Literature of Foreign Body Reaction with Stellate Inclusions in Giant Cells

No. of cases	Type of material	Author
2	Oil (aspiration)	Hirsch[9]
4	Paraffin (injection)	De Buck and Broeckaert,[4] Hirsch,[9] Firket[17]
7	Catgut suture	Hirsch[9]
6	Sebaceous material, hair, desquamated epithelial	Goldmann,[1] Herxheimer and Roth,[8] Hirsch[9]
2	Retained secretion (breast)	Letulle,[6] Hirsch[9]
1	Asbestos	Skavlem and Ritterhoff[18]*
8	Necrotic tissue, cholesterol crystals, old hemorrhage	Hirsch,[9] Firket[17]
2	Ectopic fat	Hirsch[9]

* Coexistent sarcoidosis.

the etiologic agent. The other 7 cases were diagnosed as either sarcoidosis or granulomatous inflammation of unknown etiology. In the literature it is possible to find 21 cases in which this tissue reaction is seen in association with giant cells containing stellate inclusions. These are listed in Table IV.

It is of interest to note that of the 21 cases of granulomatous inflammation in the literature, 12 were diagnosed as sarcoidosis; and that of the 8 cases in my material, 4 were so labeled. This diagnosis in most instances rests on histologic and clinical findings of doubtful specificity. The histologic basis for the diagnosis of sarcoidosis depends on minor variations in structure of the tubercle-like granuloma. Attempts have been made by Robb-Smith [11] and Dutra [12] to differentiate certain of these granulomas on morphologic grounds. Such features as the absence of necrosis, degree of fibrosis, size of the giant cells and their position in the granuloma, the distribution of reticulum, and the presence or absence of stellate inclusions have been suggested as useful in separating Stengel-Wolbach's sclerosis and beryllium granulomatosis from sarcoidosis. Lever and Freiman [13] and Kay,[14] using the features

suggested by Robb-Smith, were unable to distinguish between Stengel-Wolbach's sclerosis and sarcoidosis, and in fact concluded that these are the same disease. More recently Silverman and Erickson [15] concluded that the separation of sarcoidosis of the Darier-Roussy type and subcutaneous beryllium granulomatosis on morphologic grounds is

TABLE IV

Twenty-One Cases from the Literature of Tuberculoid Granulomatous Inflammation with Stellate Inclusions in Giant Cells

No. of cases	Organ involved	Diagnosis	Author
1	Spleen	Sarcoidosis	Friedman[10]
4	Spleen	Sarcoidosis	Kay[14]
1	Lung	Sarcoidosis	Nickerson[22]
1	Skin	Sarcoidosis	Lever and Freiman[13]
1	Skin	Sarcoidosis	Winkler[26]
1	Skin	Lupus pernio	Herxheimer and Roth[8]
1	Skin	Sarcoidosis	Jadassohn[19]
1	Skin	Foreign body granuloma	Diss[20]
1	Skin	Sarcoid (Darier-Roussy)	Görl[21]
1	Skin	Tuberculosis	Herxheimer and Roth[8]
1	Spleen, liver, lymph nodes	?	Wolbach[2]
1	Lungs, spleen, liver	?	Wolbach[2]
1	Lungs, lymph nodes	Oil aspiration	Hirsch[9]
1	Parabronchial lymph nodes	Tuberculosis	Hirsch[9]
1	Lungs	Bronchiolitis obliterans	Frothingham[23]
2	Spleen	Sarcoidosis(?)	Mallory[24]
1	Lungs	Sarcoidosis and asbestosis	Skavlem and Ritterhoff[18]

highly dubious. It is extremely doubtful in my opinion whether the lesions of tuberculoid leprosy, productive tuberculosis, beryllium granulomatosis, sarcoidosis, some of the fungous granulomas, and other granulomas produced by certain lipids can be separated on morphologic grounds when an etiologic agent is not demonstrable. Furthermore, it is of interest that not only the histologic lesion, but also the organ distribution of systemic sarcoidosis is simulated by beryllium poisoning and by oil aspiration. Pinkerton [16] and Hirsch [9] have shown

that aspirated oils are carried in the lymphatics and produce tubercle-like granulomas, not only in the lungs, but also in the lymph nodes, liver, and spleen. Hirsch's case of oil aspiration showed stellate inclusions in the giant cells. The frequent association of stellate inclusions with foreign or ectopic material suggests that at least some cases diagnosed as sarcoidosis are examples of a non-specific inflammatory granulomatous response to any one of many local or lymphatic circulating agents or substances. Proof of this will have to await more exacting attempts to demonstrate the agent in each case.

The third type of reaction, acute and chronic inflammation and repair, differs in only minor details from the foreign body reaction. Five cases in the present material are listed under this heading. These show giant cells with inclusions and varying degrees of acute and chronic inflammation. In some lymph nodes the picture is that of hyaline scarring with giant cells containing inclusions in the adjacent lymphoreticular tissue. Nine similar cases are found in the literature and are listed in Table V.

TABLE V

Nine Cases from the Literature of Acute and Chronic Inflammation and Repair with Stellate Inclusions in Giant Cells

No. of cases	Organ involved	Diagnosis	Author
1	Lungs	Adenocarcinoma, colon	Wolbach[2]
1	Lungs	Pernicious anemia	Wolbach[2]
1	Lungs	Bronchiolitis fibrosa obliterans	Amoroso and McNally[25]
1	Lungs	Thyroidectomy (post-operative death)	Hirsch[9]
1	Lungs	Pernicious anemia	Hirsch[9]
1	Lungs	Bronchiolitis obliterans	Vogel[5]
1	Bronchial lymph node	Carcinoma of nasopharynx	Wolbach[2]
1	Uterus	Hydrosalpinx	Hirsch[9]
1	Os lunatum	Old fracture	Hirsch[9]

In the 5 cases in my study, this reaction is found in the lungs in 2 cases and in tracheal and peribronchial lymph nodes in 2 others. Of the cases listed in Table V, this reaction is found in the lungs in 6 and in a bronchial lymph node in one other case. No foreign or ectopic material was demonstrated in any of these cases, but the relatively ready access of all types of aspirated material to the lungs suggests

that at least some of these cases could be grouped as foreign body reactions.

Review of the literature appears to validate the separation of the tissue reactions associated with stellate inclusions in giant cells into three types. The combined data from the present series and from the literature are analyzed in Table VI.

TABLE VI

Data on Thirty-One Cases from the Present Series Combined with Those from Seventy-Five Cases in the Literature

	Foreign body reaction	Tuberculoid granulomatous inflammation	Inflammation and repair	Insufficient data	Total cases
Present series	18	8	5	0	31
Literature	32	21	9	13	75
Total	50	29	14	13	106

CONCLUSIONS

The staining reactions, resistance to weak acids and alkalis as well as to high temperatures, and the results of micro-incineration studies indicate that stellate inclusions are organic protein structures.

These inclusions are found in association with three types of tissue response; namely, foreign body reaction, tuberculoid granulomatous inflammation, and acute and chronic inflammation and repair.

The frequency of the association of these inclusions with foreign body reactions and the great diversity of the foreign bodies are strong evidence against these inclusions being specific for any disease entity.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

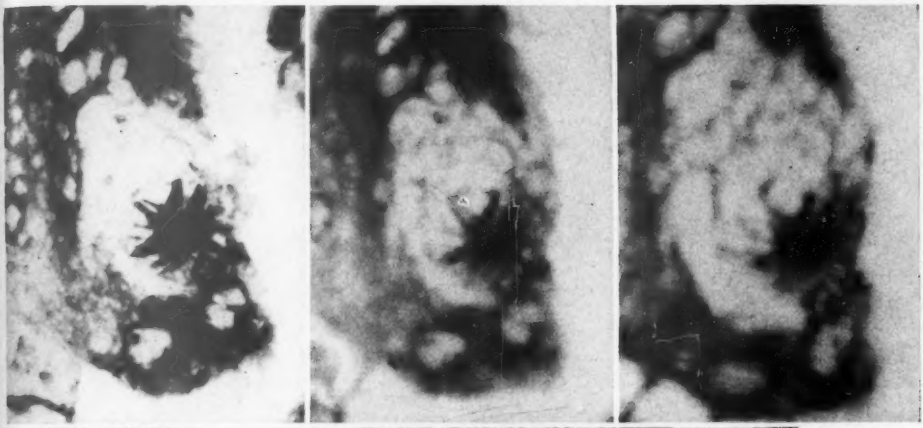
PLATE 118

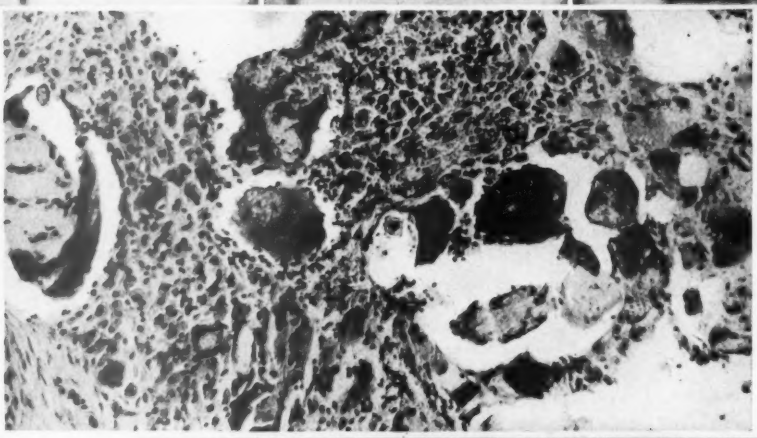
FIG. 1. (A) Control section before heating, showing giant cell with stellate inclusion. Phosphotungstic acid hematoxylin stain. (B) Same cell after heating to 250° C. Fading of the stain may be noted, with good preservation of the inclusion. (C) Same cell after heating to 350° C. The inclusion is distorted but the star shape and delicate spines are easily recognized.

FIG. 2. Case 19. From a dermoid cyst of the ovary. Foreign body reaction to ectopic hair and sebaceous material. The giant cell to the left of the center has two stellate inclusions.

FIG. 3. Case 17. Foreign body reaction to sodium urate. A giant cell near the center of the field has a stellate inclusion.

1A 1B 1C





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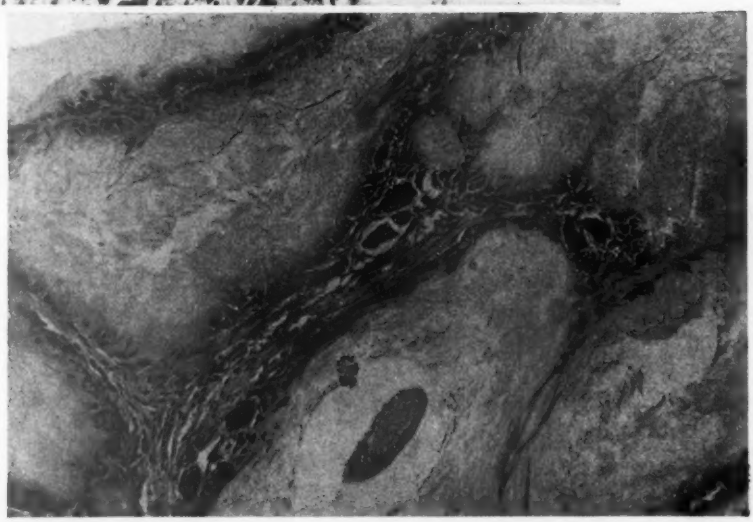


Cunningham Stellate Inclusions in Giant Cells

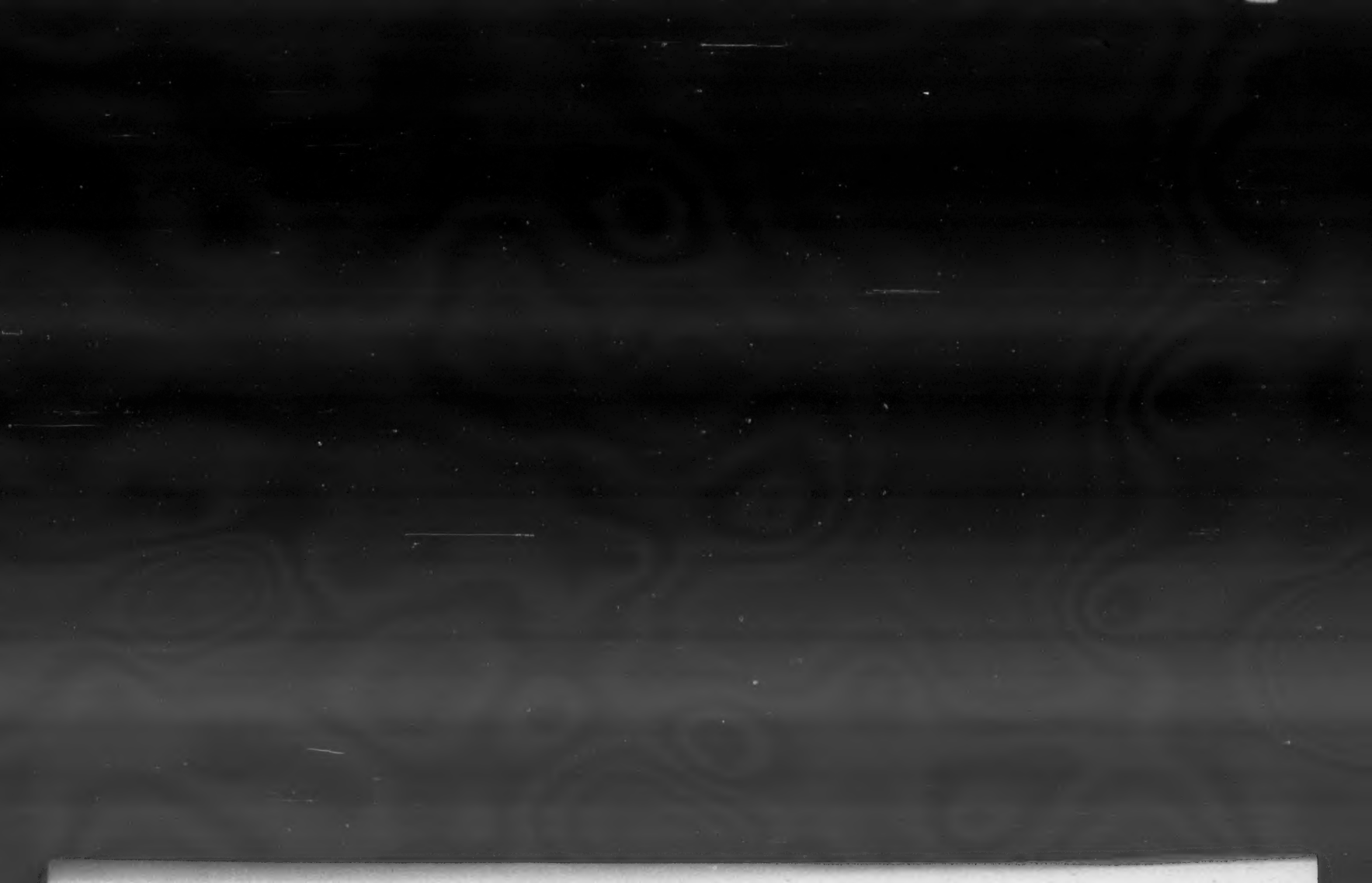
PLATE 119

FIG. 4. Case 15. Foreign body reaction to ectopic fat. The giant cell indicated by the arrow contains a stellate inclusion, shown at a higher magnification at the upper left.

FIG. 5. Case 31. Foreign body reaction around cholesterol crystals in an area of old hemorrhage. The stellate inclusion indicated by the arrow is seen in higher magnification at the upper right.

FIG. 6. Case 30. Tuberculosis(?). Granulomatous reaction with central fibrinoid necrosis. The stellate inclusion indicated by the arrow is shown at a higher magnification at the upper left.

FIG. 7. Case 21. Sarcoid. Tuberculoid granulomatous inflammation without necrosis. A stellate inclusion is seen indistinctly to the left of and above the center of the field.



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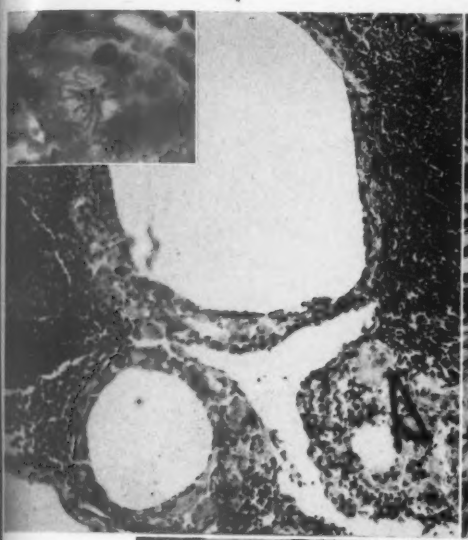

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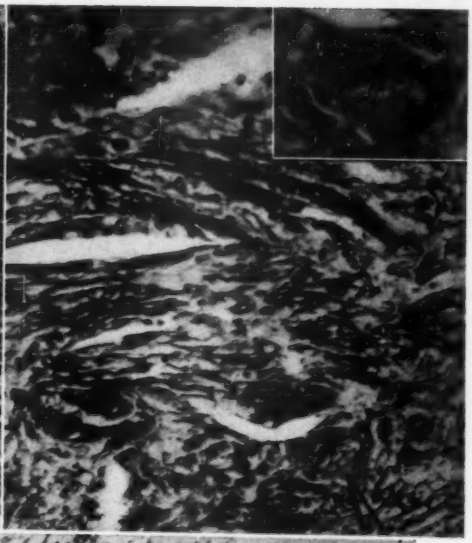

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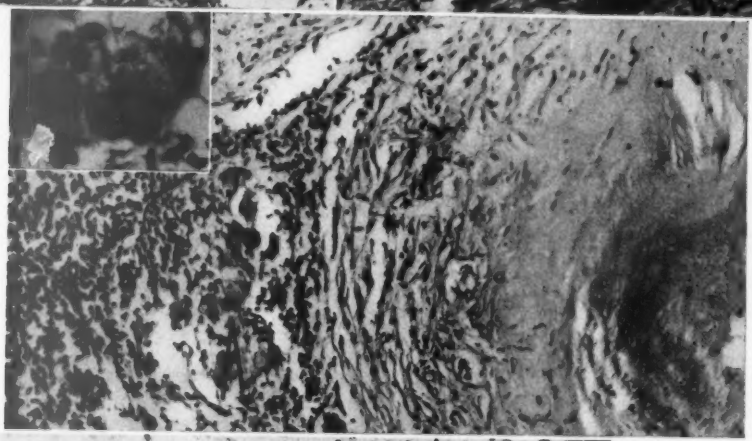

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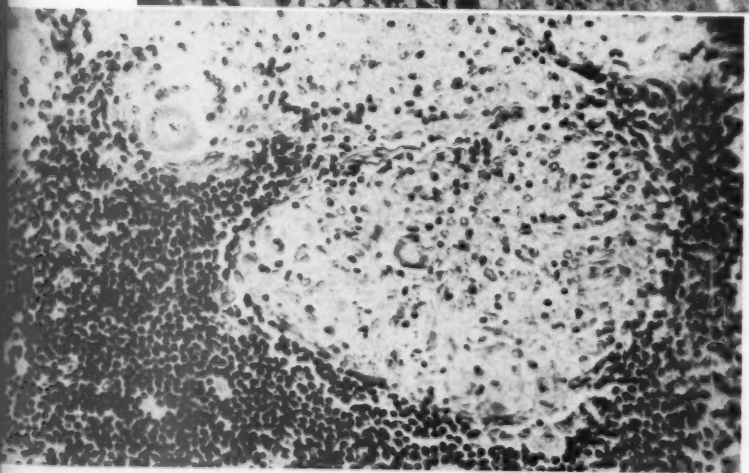
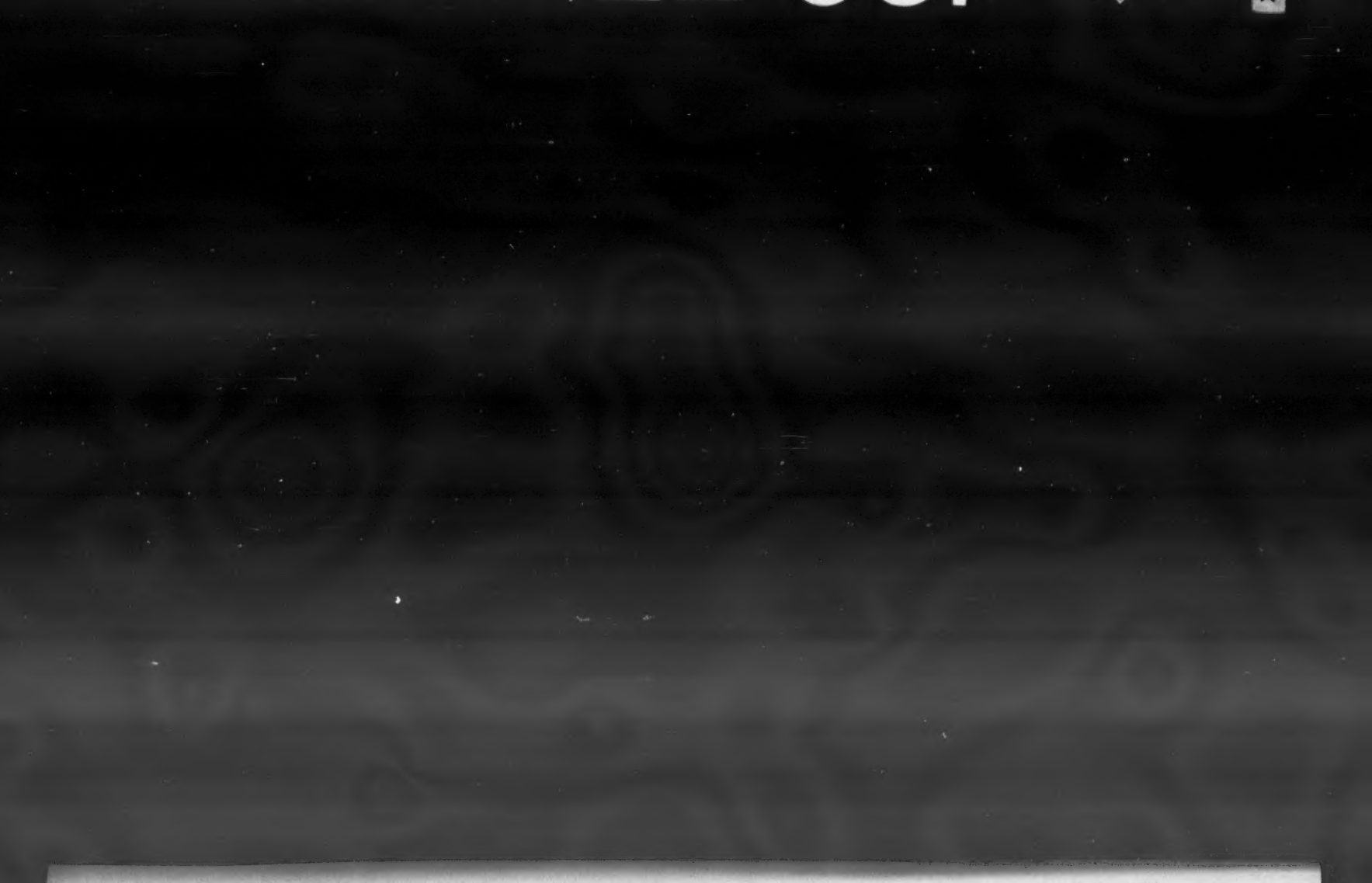


PLATE 120

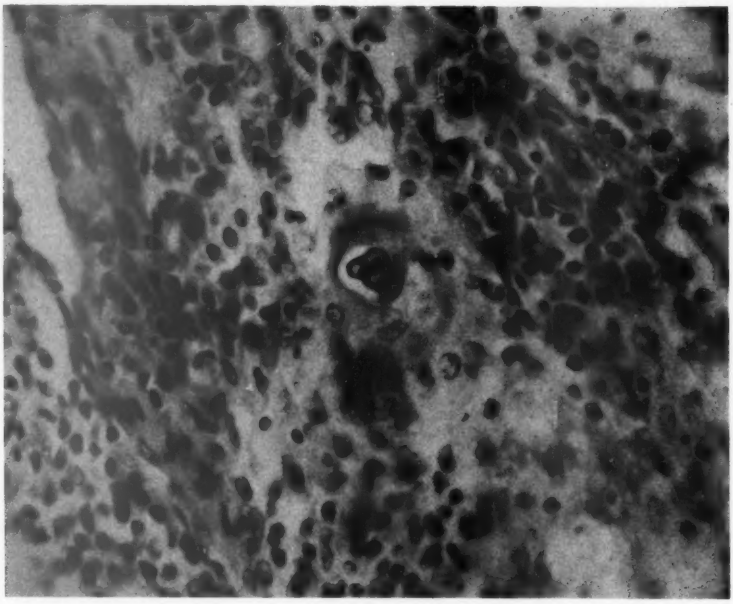
FIG. 8. Case 22. Sarcoid. (A) Multinucleated giant cell with laminated (Schaumann) body. (B) Tuberculoid granulomatous reaction and central giant cell with stellate inclusion.



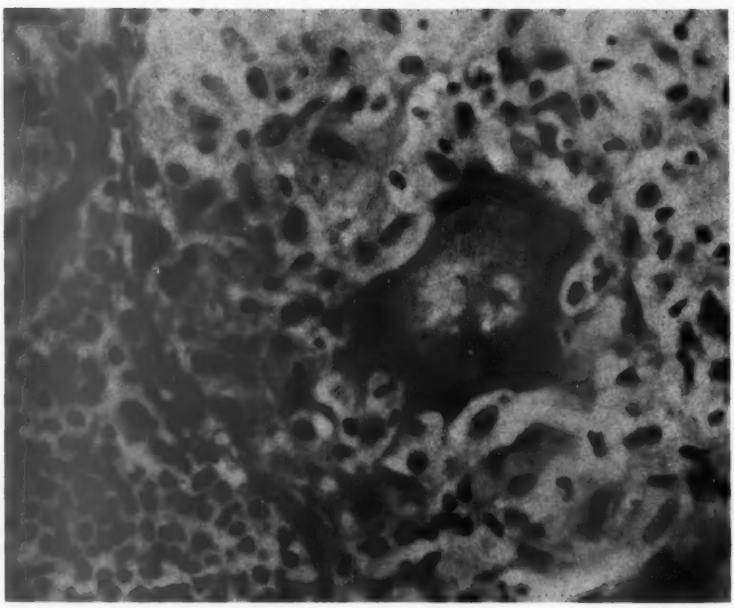
A

C

8 A



8 B

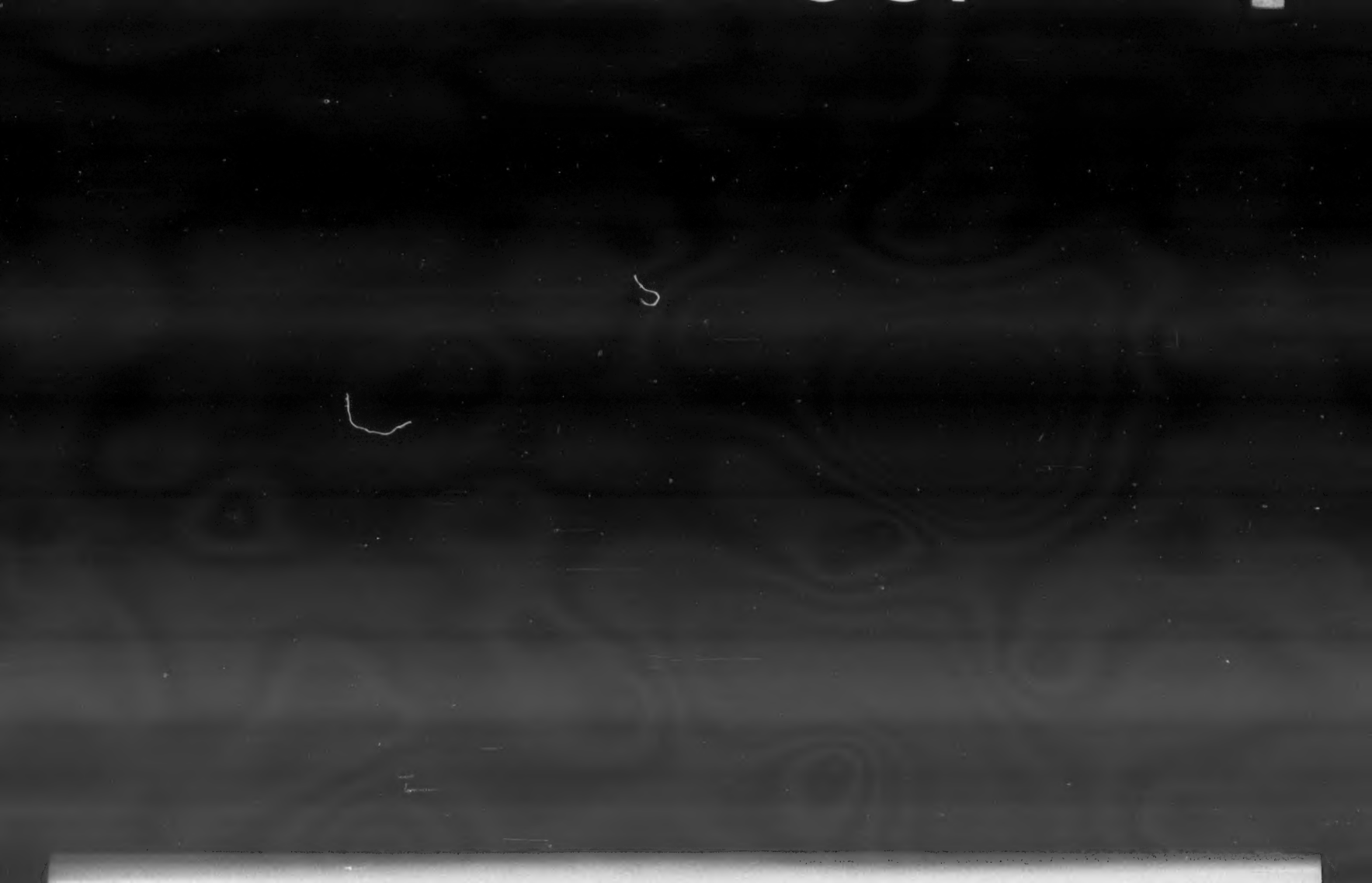


Cunningham Stellate Inclusions in Giant Cells

PLATE 121

FIG. 9. Case 23. Bronchiectasis. Perivascular chronic inflammation. Giant cell with stellate inclusion is seen at higher magnification in insert at upper left.

FIG. 10. Case 3. Small hyalinized scar in the periphery of a tracheal lymph node. Giant cell with stellate inclusion in the adjacent lympho-reticular tissue.



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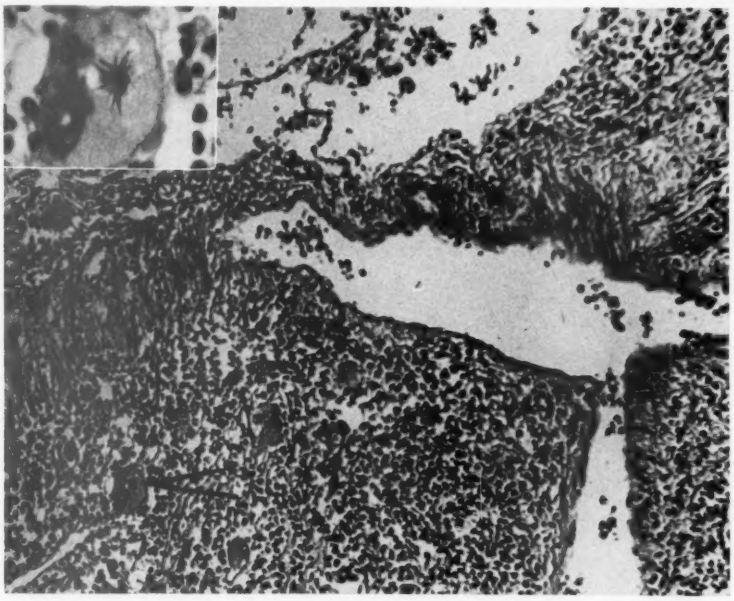

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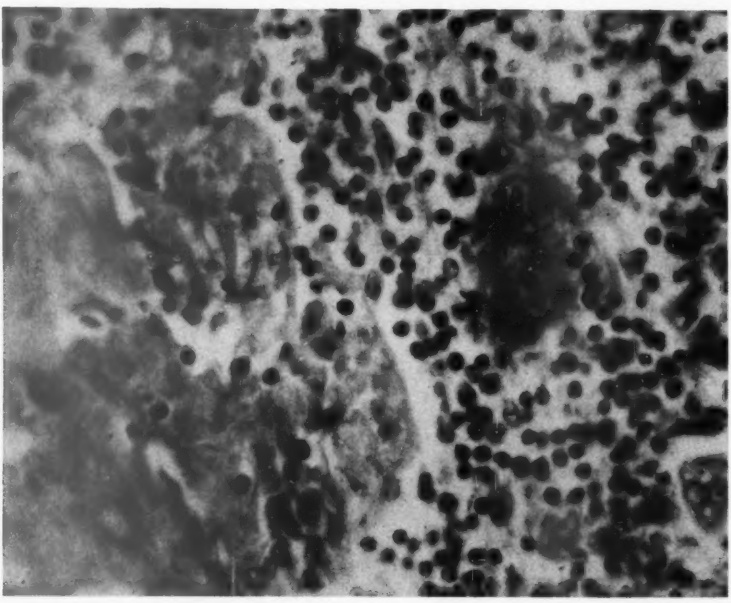

Cunningham Stellate Inclusions in Giant Cells

HISTOPLASMOSIS AND MALIGNANT LYMPHOMA *

J. F. Murray, M.D., and F. A. Brandt, B.A., B.Sc.

(From the Department of Pathology, South African Institute for Medical Research, Johannesburg, South Africa)

The first recorded case of histoplasmosis from Africa appears to be that of Simson and Barnetson [1] in 1942. At the time of publication their patient appeared to be maintaining a fair state of health. Subsequently, however, his condition deteriorated and he died a few months later. Permission for necropsy was not obtained.

Despite the rarity of the condition, it is interesting to note the many clinical aspects from which the subject has been approached. It has been considered as a pediatric problem [2]; as part of the differential diagnosis of pulmonary calcification,[3] of otolaryngeal conditions,[4] of oral lesions in dentistry,[5] of genital lesions,[6] and of dermatologic and mucosal ulcerative lesions [7]; and as a condition associated with lymphoblastoma.[8]

A recent review [7] referred to 88 cases in the literature and added a further case. In a perusal of the literature we have found 42 other case records.[6,9-26] Of the total of 131 cases only 3 occurred in Africa.[1,15] It seems appropriate, therefore, to record 3 additional cases which have been observed in this laboratory. A further reason for placing them on record lies in the striking resemblance which one of them bore to malignant lymphoma. Cawley and Curtis,[8] reviewing the same 88 cases as Miller *et al.*,[7] found 4 in which a concomitant lymphoblastoma existed. Our series includes a fifth such case and our survey of the literature revealed one other, making a total of 6.

Several authors, including Miller *et al.*,[7] also have pointed out the association of tuberculosis and histoplasmosis. One of the 4 cases observed in this laboratory showed a similar association of diseases.

Report of Cases

Case 1

Addington Hospital R.N. 6208 and 7622/44. The patient, a handyman by occupation, was a European male, 59 years old, who was admitted to Addington Hospital, Durban, on July 6, 1944. The available hospital notes indicate that he was complaining of ulceration of the palate and lower lip.

On examination two ulcers were noted on the lower lip, one near either angle of the mouth. Each ulcer was 6 mm. in diameter. The whole lesion was raised above the surface of the lip but the edges were not heaped or rolled, the center of the ulcer being in the same plane as the edges. There was very little surrounding induration. The center was gray and necrotic. In addition there was a large, irregular ulcer on the left side of the soft palate involving the left faucial pillar. The edge was slightly

* Received for publication, October 13, 1950.

raised but was not rolled or everted. The center of the ulcer was necrotic and showed multiple, small, hemorrhagic points. There was no induration of the surrounding tissue. Two other ulcers, 6 mm. in diameter, were present in the midline and on the right side of the hard palate. They resembled the ulcers on the lip. Multiple petechiae were present on the hard palate.

There were no other significant findings on physical examination. In particular, there was no lymphadenopathy, splenomegaly, or hepatomegaly. No report on roentgenograms of the chest was available. The patient was febrile throughout his illness.

Laboratory examinations showed the hemoglobin to be 79 per cent; erythrocytes, 4,500,000; leukocytes, 4,500; Wassermann reaction was negative; sputum contained acid and alcohol fast bacilli on one occasion but was negative on five subsequent occasions; blood culture, negative (observed for only 5 days); urine, a trace of albumin. Tissue taken for biopsy of the ulcer of the hard palate showed the presence of *Histoplasma capsulatum*.

The patient was treated with 30 gr. of potassium iodide every 6 hours and with 1 gm. of M. & B. 760 * every 4 hours. He also received 1 gr. of antimony tartrate on alternate days to a total of 12 gr., and a course of sulfadiazine. The general clinical condition did not change significantly except that the patient grew progressively weaker and continued to lose weight. He was discharged from the hospital for short periods but died soon after his final readmission on October 15, 1944.

Necropsy was carried out on the same day when, in addition to the labial and buccal ulcers, it was found that the retropharynx was partially obstructed by large papillomatous growths. The tongue showed a large ulcer involving its posterior third. The epiglottis was destroyed and the vocal cords were involved by a similar process. The lungs showed areas of tuberculous consolidation at the left apex with cavitation and the appearance of bronchial spread. The colon showed areas of ulceration. No other significant pathologic findings were recorded.

Specimens from the suprarenals, colon, lungs, hilar glands, liver, and tongue were submitted for histologic examination. The sections from suprarenals, colon, and tongue showed typical lesions of histoplasmosis with vast numbers of reticulo-endothelial cells, the cytoplasm of which was packed with yeast bodies. Sections of the lungs showed old fibrosing tuberculosis in which acid-fast bacilli were observed. No organisms of histoplasmosis were found. The liver showed congestion, fatty degeneration, and the presence of intracellular deposits of iron-containing pigment, but no histologic evidence of histoplasmosis was found and the sections were negative for yeast bodies. Sections of the hilar lymph glands showed no evidence of histoplasmosis. Splenic tissue was not submitted.

Case 2

J. G. was a European male, 64 years of age, who was retired but had been a clerk accountant. The patient was admitted to Addington Hospital, Durban, on April 1, 1947, under the care of Dr. Stafford Mayer. During the previous 4 months he had noticed that his tongue was brightly colored and was becoming swollen. At first it did not inconvenience him but in March the tongue became painful and he was unable

* M. & B. 760 is a sulfathiozole preparation made by May & Baker, Dagenham, England.

to eat with comfort. There was no history of trauma to the tongue. During the 3 months preceding his admission he had lost considerable weight.

On admission the patient was observed to be a well built man who had obviously lost considerable weight. His speech was thick. His tongue was enlarged to twice its normal size and was purplish red. A superficial erosion involved the posterior two-thirds of the tongue. The ulceration was superficial with some heaping up of tissues in some parts and with fissures in other parts (Fig. 1). There was some underlying induration of the tongue. Only very slight tenderness was elicited. No enlarged cervical glands were found.

A specimen from the ulcerated area of the tongue was obtained for biopsy on April 3, and section showed the presence of histoplasmosis. Scrapings of the tongue were successfully cultured for *H. capsulatum*. Laboratory investigations showed a negative Wassermann test; hemoglobin, 62 per cent; red blood cells, 3,580,000; white blood cells, 5,200; polymorphonuclear cells, 86 per cent; large mononuclear cells, 4 per cent; lymphocytes, 10 per cent; sedimentation rate, 45 mm. per hour; serum proteins, 6.3 gm. The urine showed a faint trace of albumin. A roentgenogram of the chest was normal.

Various methods of treatment were used, including potassium iodide; penicillin (100,000 units every 3 hours for 2 weeks—a total of 11 million units), streptomycin (as the drug was in short supply only 9 gm. could be given), sulfadiazine, and novarsenobillon.*

In spite of treatment, the appearance of the tongue remained unchanged and the pain gradually increased. Eating became a problem and the patient had to be coaxed to drink even small quantities. It was finally decided to do a gastrostomy. The operation was carried out June 18 under local anesthesia. Postoperative hiccoughs developed and continued in spite of all treatment (including a left phrenic crush). The patient gradually weakened and died on the tenth postoperative day.

At post-mortem examination on June 29, lobar pneumonia was found on the left side. The spleen and liver were enlarged but, while the former was soft, the latter showed a fine cirrhosis. The right suprarenal gland was replaced by caseous tissue. Apart from these findings and those which had been noted on the tongue during life, no significant pathologic findings were observed. The gastrostomy opening appeared to be healthy.

Microscopic examination of the tissues submitted from tongue, hilar and mesenteric lymph glands, bone marrow, liver, spleen, lungs, and suprarenal gland was carried out. The tongue and right suprarenal gland showed histoplasmosis, with numerous yeast bodies. Sections of the lungs showed interstitial fibrosis associated with the histologic features of chronic tuberculosis although no acid-fast bacilli were found. No yeast bodies were found in the sections of lung tissue. The bone marrow showed no significant change. The site of origin of the marrow was not stated in the particulars supplied. The hilar and mesenteric lymph glands showed no evidence of histoplasmosis. The liver showed an early perilobular fibrosis and one or two very small foci of reticuloendothelial reaction with follicle formation and occasional multinu-

* A spirochetocide made by May & Baker, Dagenham, England.

cleated giant cells of the Langhans type. Neither acid-fast bacilli nor yeast bodies were found in the foci of reticulo-endothelial reaction or elsewhere in the liver. The splenic tissue showed some fibrosis of the pulp and scanty reticulo-endothelial follicles. The latter showed central necrosis and were bordered by multinucleated giant cells. Neither tubercle bacilli nor yeast bodies could be found in the follicles.

Case 3

The patient, B. T., a European male, 13 years old, had lived all his life in Southern Rhodesia. Until the age of 8 years he had been in good health. At that time an enlarged gland appeared in the left side of the neck. General health was unaffected and the child led a normal life. The gland slowly enlarged and others appeared until, at the age of 12 years, it was decided to remove them surgically. At that time it was found that the submental deep cervical group and the glands in the posterior triangle of the left side of the neck were all enlarged. Slight anemia was said to be present (figures not available) and a tentative diagnosis of "Hodgkin's disease, or some analogous disorder demonstrating itself by widespread lymph gland hyperplasia, not tuberculous" was made. On March 12, 1948, under general anesthesia, "all enlarged and palpable glands in the left cervical region were removed. For the most part these glands formed a continuous chain from the left submental region to the thoracic inlet and posteriorly and laterally to Sibson's fascia. Thereafter some isolated glands were found and removed so that none were apparently left. In bulk the removed glands added up to the dimensions of a small closed fist."

The glands were submitted for histologic examination and the pathologist reported as follows: "The specimen consists of numerous well defined enlarged lymph glands varying in size from that of a pea to that of a golf ball. The consistence is firm and the cut surface is homogeneous and pale. There is no necrosis. Histologic examination shows complete replacement of the normal architecture by a proliferation of mature lymphocytes. There is no persistence of the follicular arrangement of these cells. This finding is common to all of the glands that were sectioned but, in addition, in some of them, there are small isolated giant cell systems with features resembling tuberculosis. Tubercle bacilli were not, however, demonstrable in these lesions. The features are those of primary lymphocytic lymphoma (or reticulosis) with superadded foci of tuberculosis."

A blood count at the same time showed the hemoglobin to be 88 per cent; red blood cells, 4,800,000; white blood cells, 10,500; packed corpuscular volume, 43 per cent; polymorphonuclear cells, 35 per cent; large monocytes, 1 per cent; lymphocytes, 59 per cent; eosinophils, 4.5 per cent; basophils, 0.5 per cent. No immature leukocytes were observed in the stained films.

At about the same date a skin eruption (eczema?) appeared and continued at intervals thereafter. It varied from an occasional focus to an extensive moist dermatitis. A dermatologist subsequently diagnosed atopic eczema. This was confirmed by histologic examination and no fungi or other causative factors were found in the sections then (December 7, 1948) or on later review. Two months after the surgical excision of the glands other glandular swellings appeared on the same side of the neck but above the surgical scar. These glands steadily enlarged.

Six months later (December, 1948) we saw the patient for the first time, when he was sent from Rhodesia with a request for a review of the diagnosis. By this time he had developed large glands in the left supraclavicular and upper cervical regions. A very large gland was present also in the left axilla. One or two very small, shotty glands were palpated in the right side of the neck but none elsewhere. The spleen was slightly enlarged but no abdominal glands or evidence of hepatomegaly could be

found. The patient was afebrile. He felt and looked healthy except for the lymphadenopathy. A radiograph of the chest was negative, with neither calcifications nor enlarged hilar shadows. Apart from eosinophilia of 15.5 per cent (1,040 per cmm.), the blood picture was normal. A similar eosinophilia had been noted in Rhodesia subsequent to his operation and was thought to be associated with the skin condition. The bone marrow (December 7, 1948) showed no abnormalities and on review of the slides at a later date no organisms of histoplasmosis were found. The marrow was not cultured at the time of aspiration as the correct diagnosis was not then suspected and the marrow was examined with the diagnosis of malignant reticulosis in mind. Serum proteins were albumin (3.8 gm.) and globulin (1.7 gm.) per 100 ml.

No evidence of lymphatic leukemia or lymphosarcoma having been found in the blood or marrow, we requested that another gland be removed for biopsy. This was refused by the parents, but the pathologist who had examined the gland removed 6 months previously kindly permitted us to see the original sections. It was found that, as stated in the original report, there was a marked lymphoid hyperplasia which, at first sight, resembled lymphatic leukemia (Fig. 2). The foci of reticulo-endothelial reaction, which had been reported, were again observed (Fig. 3) and, although they resembled the lesions of tuberculosis, a search for acid-fast bacilli was fruitless. However, in the cytoplasm of the reticulo-endothelial cells adjacent to the follicles numerous yeast-like bodies having the characteristics of *H. capsulatum* were found (Fig. 4). In view of this, a diagnosis of histoplasmosis was made. A histoplasmin skin test was negative.

Following the establishment of the diagnosis, the patient returned to Rhodesia where, in spite of enlarging glands and spleen, he remained subjectively well for a further 8 months. After July, 1949, his general condition began to deteriorate. He developed a normochromic, normocytic anemia (hemoglobin, 10.9 gm. per cent; erythrocytes, 4 millions; leukocytes, 15,300; neutrophils, 59 per cent; monocytes, 7 per cent; lymphocytes, 33 per cent; eosinophils, 1 per cent). The glandular swelling on the left side of the neck began to fluctuate. A needle puncture produced pus in which abundant yeast forms of Histoplasma were demonstrable (Fig. 5). Cultures and biologic tests carried out by Dr. B. Tulloch of Bulawayo confirmed the identity of the organism.

When last seen by us (October 23, 1949) the patient's condition had deteriorated dramatically. He was cachectic, thin, and emaciated, with a constant swinging temperature of 99° to 103° F. Large glands were palpable on both sides of the neck. Those on the left had ulcerated and were discharging abundant thin pus from a large fungating mass of granulation tissue. In addition, large, soft swellings were visible over the right sternoclavicular joint, just below the midpoint of the left clavicle, and in the left axilla. A palpable gland was present also in the right groin. The spleen was palpable 3.8 cm. below the costal margin and the smooth, sharp edge of the liver could just be felt. No buccal or laryngeal lesions were found and, except for the ulceration in the left cervical region, the skin was unaffected. Sulfonamides, streptomycin, penicillin, chloromycetin, and aureomycin were administered successively but none of them had any effect on the temperature or steadily downward course. In the following month (November, 1949) the histoplasmin skin test was repeated but was again negative although good positive results were obtained with the same material in control subjects. In December, 1949, the parents decided to take the patient overseas for further advice. He died a few days after his arrival, 6 years after the first evidence of lymphadenopathy and 1 year after the diagnosis of histoplasmosis was established.

At necropsy (performed by Dr. G. J. Cunningham of St. Bartholomew's Hospital) the following were the pertinent findings. The body was pale, thin and wasted. Two large (2 cm. and 4 cm. in diameter)

and one small ulcer (0.5 cm.) were present, overlying and penetrating the left sternomastoid muscle. A healed scar was found in the left axilla and a superficial abscess overlay the pectoralis major muscle. On each side of the neck there was a chain of enlarged deep lymph nodes. They were matted and showed variation in appearance, some being hemorrhagic, some whitish in appearance, and some yellow and necrotic. A further chain of similar lymph nodes extended along the thoracic aorta. In addition, there was much enlargement of the nodes at the root of the lung and many of these were necrotic and in process of breaking down.

On the right side of the neck, 8 cm. below the level of the upper border of the thyroid cartilage, was a cavity 2.5 cm. in diameter, lined by yellowish necrotic material and containing the projecting end of a blood vessel, the lumen of which appeared obliterated. Bounded externally by enlarged lymph nodes, the cavity lay between the junction of the left and right innominate veins and in close relation to them. The superior vena cava showed a slightly reddened intimal area close to the junction with the subclavian, but in none of these veins was any evidence of thrombosis found. On the left side of the neck in close relation to the origin of the subclavian vein was a cavity similar to the one described above. The left common carotid artery, although passing through a mass of inflamed tissue, showed no abnormality.

A mass of lymph nodes posterior to the superior vena cava and anterior to the right main bronchus, had undergone necrosis and ulcerated into the latter structure. The tracheal mucosa and that of the main bronchi was reddened, and the lumen contained much necrotic material. The left *axillary lymph glands* constituted a large mass.

Both *lungs* contained a number of small yellowish nodules scattered throughout their substance. In addition there was basal congestion and many subpleural solid dark areas had the appearance of infarcts. Four ulcers (1.5 cm. in diameter) were present at the lower end of the *ileum* in close relation to the ileocecal valve. Many smaller superficial ulcers could be seen in lymphoid follicles in the neighborhood and in the *cecum*.

All groups of *abdominal lymph glands* were enlarged. The *liver* was slightly enlarged, pale and fatty. The *spleen* was greatly enlarged (680 gm.). It was dark in appearance but no obvious lesions were present. The *kidneys* showed one or two areas suggestive of necrotic lesions. The stripped surface was studded with hemorrhages. The *suprarenal glands* (right, 10 gm.; left, 12 gm.) were normal.

The principal *histologic features* were as follows: *Spleen.* Considerable congestion of the pulp was noted, with a number of scattered

giant cells resembling those seen in tuberculosis. These cells and the majority of the reticulo-endothelial cells of the pulp contained large numbers of *H. capsulatum*. In addition a few hemosiderin-containing macrophages were seen. *Lung*. General congestion was present, with fibrinous exudate on the pleural surface. There were some large foci of caseous material. In addition, numerous small lesions consisted of collections of epithelioid cells and multinucleate giant cells. These lesions were related chiefly to blood vessels in the interstitial tissue and some of the epithelioid cells contained a few parasites. The solid areas observed subpleurally were infarcts in which clumps of organisms could be seen. *Intestine*. Ulceration extended to the muscle layer. The ulcer base was composed of epithelioid cells in large numbers and of giant cells. In cells of both types numerous parasites could be seen. *Lymph node*. Usual architecture was replaced by granulation tissue and areas of caseation necrosis. Giant cells and epithelioid cells were present in enormous numbers, and most of these contained large numbers of parasites.

Discussion

Our first 2 cases were straightforward examples of histoplasmosis and, although one of them (case 1) was complicated by tuberculosis, they showed no features which have not been described previously in the literature. Our interest has centered mainly on case 3 which, not only clinically but also histologically, suggested a malignant reticulosis. Of the 5 such cases recorded in the literature, 3 have shown histologic evidence of Hodgkin's disease [7,9,27] and one of lymphatic leukemia.[28] The remaining case [29] was considered clinically to be lymphatic leukemia, but the only histologic examination reported is that of the skin and it was regarded by the author as showing leukemia and a fungus infection. Our case 3 was diagnosed by a competent histopathologist as a lymphoid lymphoma. It was only on review of the section that *H. capsulatum* was found. Similarly, in the case of Miller *et al.*[7] which had been diagnosed as Hodgkin's disease in 1938, scanty organisms of histoplasmosis were found on review of the section 5 years later.

The association of histoplasmosis and of a histologic picture resembling malignant reticulosis in 6 of 134 cases makes the association of these two conditions a matter of unusual interest. There would appear to be four possible explanations of the association: (1) coincidental dual affections, (2) predisposition to fungus infection as a result of an existing malignant reticulosis, (3) development of malignant reticulosis as a result of fungus infection of the reticulo-endothelial system, or (4) a cellular reaction of the reticulo-endothelial system which closely resembles malignant lymphoma.

Cases Showing Histologic Evidence of Malignant Lymphoma

Riehl's case,[29] which was the first of the 6 reported, undoubtedly showed histologic evidence of histoplasmosis in the skin section. Clinically, there were no features which were not explicable on the basis of that diagnosis. The blood count showed a slight lymphocytosis on three occasions but leukocytosis was present only once and immature cells were not reported. On one occasion eosinophils (32 per cent) were present in the peripheral blood. The diagnosis of lymphatic leukemia appears to be based on the mononuclear cellular infiltration of the skin found at necropsy. Such infiltration is a well known feature of many dermatologic conditions, including chronic inflammation, and is by no means pathognomonic of leukemia. Unfortunately, no other organs were sectioned after necropsy and, as the bone marrow had not been examined during life owing to the absence of any simple aspiration technic at that time, there is no unequivocal evidence that this patient suffered from lymphatic leukemia. A review of the clinical and necropsy findings shows no feature which could not be explained on the basis of histoplasmosis with an associated lymphocytic infiltration of the skin.

The case reported by Williams and Cromartie [28] was more adequately investigated. Sections of bone marrow, spleen, liver, and lymph gland, taken at necropsy, all showed evidence of chronic lymphatic leukemia. During life, however, the peripheral blood showed only a moderate absolute lymphocytosis. The histologic features of the lymph gland described by Williams and Cromartie correspond exactly with those found in our case 3, and yet our case showed no evidence of leukemia in a marrow specimen examined during life and no histologic changes suggestive of a malignant reticulosis are recorded in the postmortem material.

Our case 2 showed gross histoplasmosis of one of the suprarenal glands and abundant examples of *H. capsulatum* were present in sections of that organ. No yeast bodies were found in the spleen, however, which is usually heavily involved in septicemic cases. It is our opinion that failure to find the fungus in sections of proved cases of histoplasmosis does not exclude the possibility that round cell infiltrations may be the result of the histoplasmosis. The round cell infiltration observed by Williams and Cromartie [28] may have been of this nature. This seems the more likely in that our case 3, although having lymph gland lesions identical with those which Williams and Cromartie described, showed no suggestion of leukemia in other organs. In their case, Williams and Cromartie stated that "besides the lesion produced

by the parasite the lymph nodes were involved by a process morphologically identical with chronic lymphatic leukemia."

The remaining 3 cases in the literature present a slightly different problem in that the reticulo-endothelial reaction associated with the histoplasmosis was not of lymphoid type but was that of Hodgkin's disease. Parsons and Zarafonetis,[27] in describing their case G., mentioned that "In the bone marrow the lesions of Hodgkin's disease were sometimes separate and sometimes intermixed with the much more extensive lesions of histoplasmosis. . . . It is agreed by the pathologists who have seen the sections from this case that both Hodgkin's disease and histoplasmosis were present." The authors asked: "Did Hodgkin's disease, a disease in which cells of the reticulo-endothelial system become malignant, predispose this man to histoplasmosis, a disease which is characterized by the parasitization of the cells of the reticulo-endothelial system by the yeastlike form of the fungus Histoplasma capsulatum?"

In the case described by Miller *et al.*[7] both Hodgkin's disease and histoplasmosis co-existed in the earliest biopsy specimen of a cervical gland although it was not until review 5 years after its removal that the second condition was recognized in the section. At necropsy, however, Hodgkin's disease was observed in the spleen and liver. After a careful and detailed histologic description, the authors concluded that "the term Hodgkin's sarcoma best fits this pleomorphic picture." Examination of an axillary gland obtained for biopsy showed "an occasional macrophage . . . morphologically identical with the so-called Sternberg-Reed cell seen in Hodgkin's disease. However," they added, "the definite chronic granulomatous pattern of this portion of the lymph node with the distinct predominance of the macrophage type of cell lent an over-all microscopic picture which in no way suggested the presence of Hodgkin's disease."

The third case of this type[9] was diagnosed as Hodgkin's disease 2 months prior to death on the basis of a lymph node biopsy. At necropsy "microscopic study. . . . showed changes consistent with Hodgkin's disease in the liver, spleen, adrenals, and lymph nodes. Within the lesions in the adrenal and liver were seen large numbers of parasites having the morphologic characteristics of *H. capsulatum*."

The histologic picture of Hodgkin's disease has been regarded as a characteristic one though it varies from case to case and has been divided by Jackson and Parker[30] into the three types of paragranuloma, granuloma, and sarcoma. In the above 3 cases emphasis is laid in each instance upon the presence of a reticulo-endothelial reaction

having the histologic features of Hodgkin's disease. At the same time, it is known that histoplasmosis is the one fungus disease which affects mainly the reticulo-endothelial system. It recently has been shown by a number of workers that specific stimulation of the reticulo-endothelial system by foreign substances may produce bizarre and unusual cytologic reactions.[31,32] In some instances the reaction closely resembles the malignant lymphomata which the Gillmans [32] described as a Hodgkin's-like sarcoma in some of their experimental animals. It will be noted that, in describing their necropsy findings, Bunnell and Furcolow [9] used the term "changes consistent with Hodgkin's disease" while Miller *et al.*[7] remarked that "the term Hodgkin's sarcoma best fits this pleomorphic picture." The latter authors emphasized in their paper the "problem of confusing the histoplasmosis tissue reaction with that of the malignant lymphomas." It seems possible to us, therefore, that the pleomorphic picture observed in the reticulo-endothelial system in some cases of histoplasmosis may be an example of the Gillmans' [33] so-called divergent differentiation while the uniform lymphoid picture observed in other cases may be an example of convergent differentiation. The varied histologic results and the pleomorphic appearance found in different animals subjected to the same stimulus by the Gillmans suggest that it is not impossible that stimulus to the reticulo-endothelial system by histoplasmosis in man may lead to differing histologic pictures. Some of these pictures may, as they usually are, be pure granulomata while others may resemble the malignant lymphomata. Only a small percentage of the Gillmans' experimental animals [32] developed the histologic features of a malignant reticulosis, although all gradations between normal and malignant histologic pictures could be traced. Similarly, in only a small percentage of cases of histoplasmosis are the features of malignant reticulosis associated, but this percentage appears to be greater than could be accounted for by chance. And it seems to us that "the possibility of a relationship between them" suggested by Cawley and Curtis [8] may be that of cause and effect. What bearing this has on the pathogenesis of malignant reticulosis as a whole and on Hodgkin's disease in particular is not clear but it is possible that experimental work may elucidate this point. It is also not clear whether the histologic picture sometimes found is that of a true malignant lymphoma or of a "lymphoma-like" picture.

A further indication that the invasion of the reticulo-endothelial system by histoplasmosis may lead to a second disease is its frequent association with tuberculosis. Miller *et al.*[7] suggested that this is due to reactivation of an old tuberculous focus, but Arnold, in the discus-

sion of Curtis and Grekin's paper,[10] suggested that it may be due to a blocking of the reticulo-endothelial system. He suggested that this may be the cause of the associated tuberculosis and likened it to lepromatous cases, 50 per cent of which develop tuberculosis. Weidman, in the same discussion, referred to the association of torulosis and Hodgkin's disease.

Summary

Of 3 new cases of histoplasmosis, one resembled a malignant reticulosis histologically as well as clinically, and was so diagnosed on biopsy of a lymph gland. *Histoplasma capsulatum* was subsequently found on review of the same section. The patient ultimately became septicemic and died.

To us it appears that the association of histoplasmosis and malignant lymphoma may be explicable on the basis of a cytologic reaction of the reticulo-endothelial system to stimulus by a specific infection. Whether this means that the resultant reaction is a malignant lymphoma or merely a "lymphoma-like" picture is not clear at present.

We gratefully acknowledge the permission of the following practitioners to use their case notes: Dr. Tanchel, Superintendent of Addington Hospital, Durban (case 1); Dr. Stafford Mayer, Durban (case 2); and Dr. David, Bulawayo (case 3). We are indebted also to Dr. Bodley Scott and Dr. Cunningham of St. Bartholomew's Hospital, London, for their permission to use the necropsy record of case 3, and to Dr. Tulloch of Bulawayo for the culture from the same case.

ADDENDUM

Since completion of this paper three further references having a bearing on its subject matter have appeared in the literature. Furcolow [34] reported 6 cases of histoplasmosis one of which showed histologic features suggestive of Hodgkin's disease and he discussed the relationship of histoplasmosis to other granulomata. Raftery,[35] describing cases of subclinical histoplasmosis of the appendix, stated that 2 patients and a relative of a third subsequently developed malignant reticuloses. One of these cases showed the parasites in the nodal tissue on which a diagnosis of lymphoblastic lymphoma was made, thus resembling our case 3. Rodger, Terry, and Binford [36] presented yet another case of Hodgkin's disease complicated by generalized histoplasmosis and suggested that Hodgkin's disease causes an increased susceptibility to fungal infections.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Case 2. Superficial ulceration of the tongue.

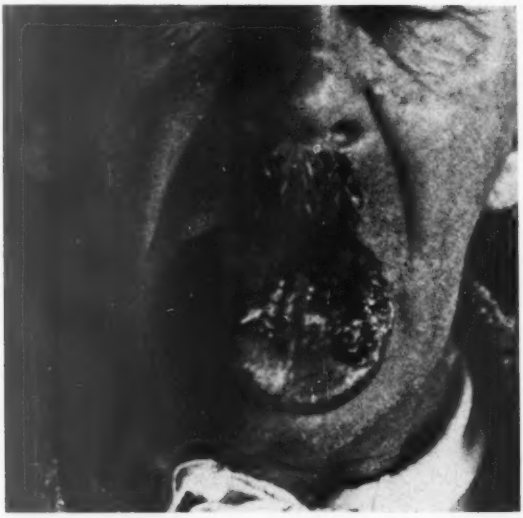
FIG. 2. Case 3. Cervical lymph node. Lymphoid hyperplasia resembles that of lymphatic leukemia. Hematoxylin and eosin stain. × 210.

A

2

M

1



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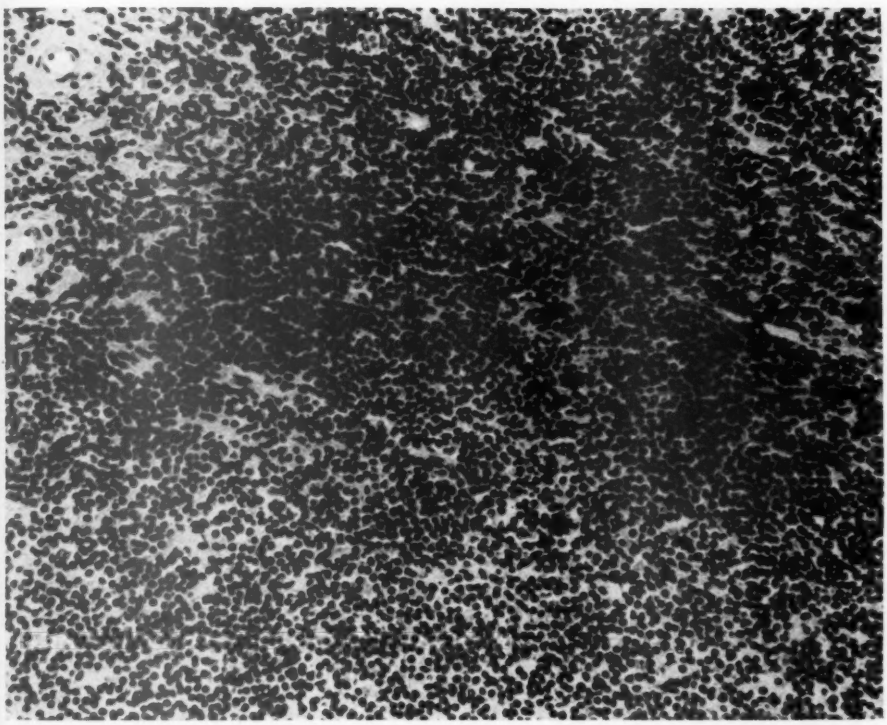

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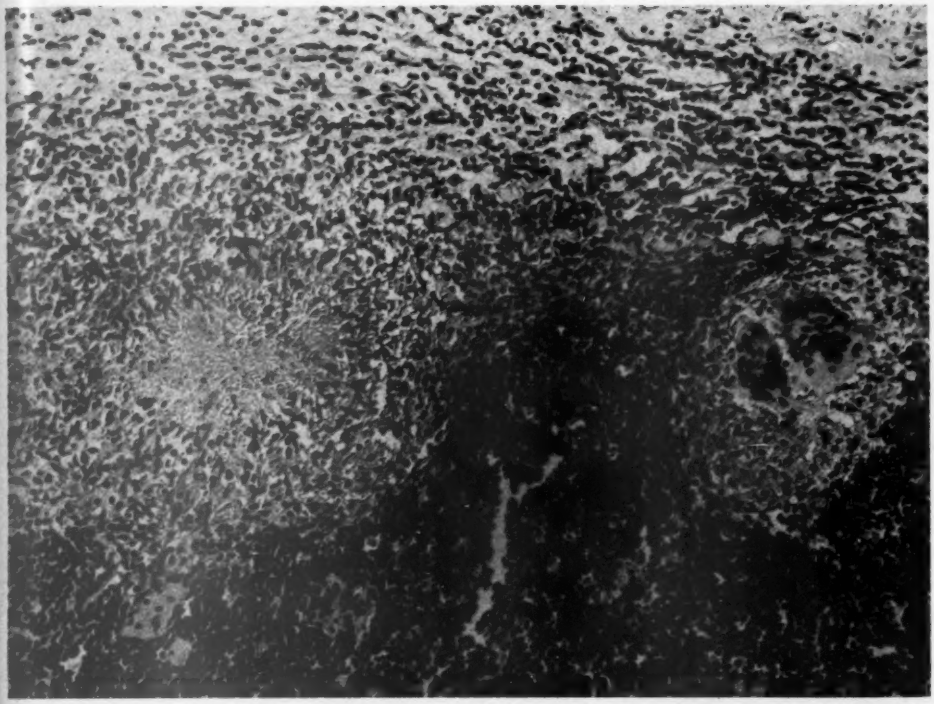
PLATE 123

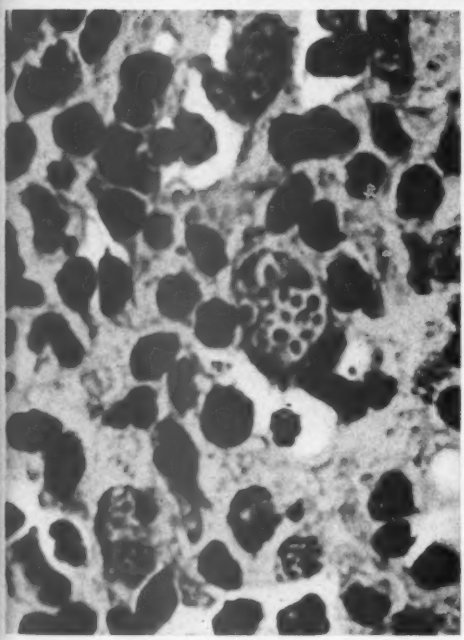
FIG. 3. Case 3. Cervical lymph gland showing foci of reticulo-endothelial reaction, associated with giant cells. Central necrosis is present in one follicle. No acid-fast bacilli were found in this section. Hematoxylin and eosin stain. × 150.

FIG. 4. Case 3. Cervical lymph gland with encapsulated yeast bodies of *Histoplasma capsulatum*. Hematoxylin and eosin stain. × 1250.

FIG. 5. Case 3. Intracellular yeast bodies in pus aspirated from a cervical abscess. Wright's stain. × 1250.

3





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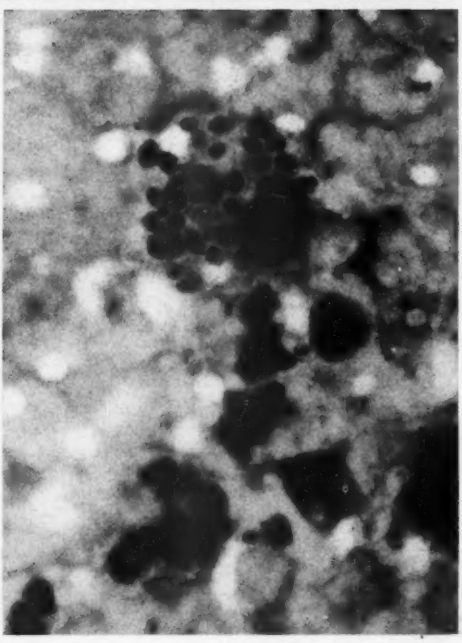

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Murray and Brandt

Histoplasmosis and Malignant Lymphoma

LYMPHOSARCOMA OF THE PROSTATE *

LESTER S. KING, M.D., and THOMAS R. COX, M.D.
(From the Pathology Laboratory of the Illinois Masonic Hospital, Chicago, Ill.)

Although malignant lymphoma arises most commonly in lymph nodes or in hematopoietic organs, primary origin from other sites, such as intestine, thyroid gland, or skin, is occasionally observed. Primary origin in the prostate gland has been considered a great rarity. The recent observation of 3 such cases in a very short period of time raises the question whether the entity is not far more common than is generally realized. Analysis of these cases raises certain problems fundamental to understanding the biology of tumors of this class.

REPORT OF CASES

Case 1

The patient was a male, 60 years old, who was first admitted for dysuria of 2 months' duration and hematuria for 4 days. The past history was non-contributory. Physical examination was negative except for grade II to grade III rubbery enlargement of the prostate. Cystoscopy showed a ragged papillary mass involving the vesical orifice and posterior urethra, appearing grossly like a carcinoma. Laboratory examination revealed moderate numbers of red and white blood cells in the urine, with 2 plus albumin. Blood count, non-protein nitrogen, blood urea nitrogen, creatinine, and blood sugar were within normal limits. Total protein was 5.85 gm. per 100 cc. Urine culture was negative. Electrocardiograms suggested myocardial damage. A transurethral resection was performed, yielding numerous linear fragments of firm, rubbery, pink-gray tissue, measuring in the aggregate 3.5 by 0.3 by 1 cm. A microscopic diagnosis of highly undifferentiated carcinoma of the prostate was made (microscopic description considered below). Acid phosphatase determinations gave 0.8 and 0.9 units on two occasions. Skeletal roentgenograms were negative. Bilateral orchiectomy was performed, and the patient discharged improved.

Six weeks later he was again admitted because of acute retention. Further transurethral resection was performed. Although the diagnosis again was made of highly undifferentiated carcinoma, the pathologic report noted that the specimen suggested some type of lymphoblastoma and recommended a trial of x-ray therapy.

Eight weeks later there was recurrence of hematuria, with pain and distention. A huge prostate, bulging into the rectum, was noted. The red blood cell count had fallen to 3.7 million, with 7.7. gm. of hemoglobin. The acid phosphatase was still only 1.1 units, but the non-protein nitrogen was 46 mg. and the creatinine 2.8 mg. per cent. Over a period of 11 days a course of x-ray irradiation was given, with a total tumor dose of 1540 r. He was discharged much improved.

Within 6 weeks, however, the tumor had again increased in size and a second course of irradiation, with a tumor dose of 1393 r., was given. Only very moderate improvement was obtained.

The patient was involved in an accident, with fracture of the leg and multiple lacerations. The subsequent course was complicated by aphasia and hemianopsia. With a tentative diagnosis resting between subdural hematoma and metastatic cancer, a subtemporal decompression was done, with negative findings. The subsequent

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course is irrelevant to the present discussion. The patient expired 1 month after the accident, 8 months after the first hospitalization and about 10 months after the first onset of urinary symptoms.

Necropsy Findings

The significant features of the necropsy examination were as follows: The scalp showed a left temporoparietal scar and decompression. Blood exuded from the urinary meatus. The testes were absent. The skin of the lower abdomen had a brownish discoloration such as occurs with a radiation reaction. Persistent thymic tissue was present in the anterior mediastinum. The thoracic organs showed only moderate basal pulmonary congestion. The spleen, weighing only 90 gm., had two tumor nodules 7 to 8 mm. across. There was extensive tumor involvement of the lymphatic system, especially in the pre-aortic nodes which were enlarged, partly confluent, homogeneous, and fleshy, with no gross evidence of necrosis. Massively enlarged nodes were present in the mesentery of the small intestine. Similar tumor overlay the right adrenal gland and was adherent to the under surface of the liver; but the liver, weighing 1600 gm., contained no tumor, while the adrenal gland was similarly not invaded. The kidneys, weighing 170 and 150 gm., respectively, were fairly well preserved, except for a moderate degree of hydronephrosis. No tumor was present in the renal substance, but tumor involved the distal part of the ureters where bilateral hydro-ureter to a moderate degree was present, more advanced on the right. The bladder and prostate formed a huge mass virtually filling the entire pelvis, and weighing together 700 gm., of which five-sixths was represented by the prostate. The bladder was small, contracted, and thick-walled, with an edematous mucosa, in large part ecchymotic and hemorrhagic. The bladder overlying the prostate showed replacement of the mucosa by irregular, fleshy, grayish white nodules appearing like a closely crowded mass of pebbles, each measuring up to 2 cm. across. These filled the trigone and surrounded the urethral orifices. The prostate was represented by a large mass, 10 by 8 cm., remarkably smooth on section, homogeneous, and grayish yellow to grayish pink. No prostatic tissue proper could be recognized. The seminal vesicles could be identified as faint cystic cavities completely surrounded by homogeneous tumor tissue. The brain weighed 1400 gm. There was slight flattening of the cerebral convolutions and narrowing of the sulci. No excessive spinal fluid was present, and the meninges throughout were delicate. Faint bulging of the brain was present in the region of the decompression (left temporal lobe). On

section, there were small areas of ecchymosis and linear zones of softening, representing the tracts of surgical needling, but no areas of nontraumatic encephalomalacia were encountered. No tumor could be identified.

Microscopic Examination. The original surgical specimen consisted of highly cellular tissue. Although this was for the most part similar to the tumor found at necropsy, portions of the surgical specimen showed extensive fibrosis, with abundant distortion of tumor elements and many cells arranged in linear columns, with some arrangements suggesting abortive acinar formation.

In the necropsy sections, the tumor in general presented a fairly uniform appearance. The tumor cells were moderately pleomorphic (Figs. 1 and 2), relatively discrete, with dark-staining, slightly irregular nuclei rich in chromatin, and scanty amphophilic or faintly basophilic cytoplasm. The characteristic areas showed no definite architectural arrangement, but the cells, while discrete, were fairly closely crowded. Light reticular stroma was present in moderate amounts, coursing between individual cells and small groups of cells. Many parts of the tumor, especially in the lymph node metastases, showed small giant cells (Fig. 4), with large, very dark-staining, bizarre nuclei, usually single, but occasionally convoluted or multiple. In many portions of the tumor, especially in the metastases, small, dark-staining, irregular cells were interspersed with larger pale elements, resembling fairly differentiated histiocytes (Figs. 3 and 4). Many of these contained phagocytized débris. Others had more abundant pale pinkish cytoplasm with larger nuclei, suggesting more primitive cell forms, closer to the primitive endothelial cell. These pale elements with relatively abundant pink-staining cytoplasm were in sharp contrast to the smaller, darker cells with dark, chromatin-rich nuclei, and indistinct cytoplasm. Only occasionally could tumor cells be observed with any syncytial arrangement of cytoplasm. Mature lymphocytes were not evident, nor could myeloid cells be observed. Mitotic figures were infrequent.

The final pathologic diagnoses on this case were: malignant lymphoma of prostate, reticulum cell type; recent operation, orchiectomy; tumor metastases to pre-aortic, mesenteric, and abdominal lymph nodes, to spleen, ureters, and seminal vesicles, and to bladder and periprostatic tissues; hydro-ureter and hydronephrosis; arteriosclerosis, generalized, moderate; arteriolosclerosis of cerebral blood vessels with clinical aphasia; recent operation—subtemporal decompression

and exploration; bronchopneumonia. Subsidiary diagnoses were: persistent thymus; fibrosis of appendix; healed tubercles of liver.

Case 2

The patient was a male, 76 years of age, who entered with acute urinary retention. For the past several years there had been increasing dysuria, and marked nocturia. Hematuria had not been present. The patient complained of epigastric pain relieved by food. Except for slight epigastric and suprapubic tenderness, physical examination was negative. There was no record of a prostatic examination. Laboratory data included red blood cells of 3.6 million; white blood cells, 12,700; 84 per cent polymorphonuclear cells; 7 per cent lymphocytes; hemoglobin, 11.3 gm. Urine showed 5 to 10 white blood cells per high-power field, otherwise negative. Non-protein nitrogen, creatinine, and blood sugar were within normal limits; serologic examination was negative. Acid phosphatase was 1.19 units. Skeletal roentgenograms were negative except for arthritic changes.

By cystoscopy, trabeculation of the bladder was noted, with many tumor shoots in the posterior urethra and bladder floor. A suprapubic prostatectomy was performed, yielding a prostate gland measuring 8 by 7 by 4.5 cm. and weighing 95 gm. One lateral lobe was approximately twice the size of the other. Characteristic nodular hyperplasia was present in part, while other portions were flat, glistening, and grayish tan on section. The microscopic diagnoses were benign nodular hyperplasia of the prostate, and highly undifferentiated carcinoma of the prostate. The notation was made that the tumor was composed of widely invasive, very primitive cells, bearing a close resemblance to primitive mesenchyma or its derivative, the reticulum cell sarcoma.

Although the patient complained of gastric discomfort and was kept on amphojel, the postoperative condition was in general satisfactory. However, a large mass was noted to be growing in the incision. Fever to 102° F. developed, with considerable dyspnea. The patient expired 5 weeks after the operation and about 6 weeks after the onset of acute symptoms.

Necropsy Findings

At necropsy, external examination was remarkable only for a midline suprapubic scar, with a 4 by 1 cm. opening into the bladder. This opening and the surrounding tissue were heavily infiltrated by pale, soft, succulent tumor. The lungs contained small tumor nodules, but the thoracic contents showed no other relevant features. The mesentery was studded with nodules 1 to 3 mm. across. Within the liver was a discrete tumor nodule, 1.5 by 2 cm. The pancreas was rubbery, with some loss of the usual lobular pattern. The pelvis was filled by a mass of tumor extending laterally into the inguinal ligaments and surrounding the hypogastric arteries. An irregular cavity about 3 by 3 by 2 cm. represented the bladder, of which the walls were infiltrated by neoplasm. A sinus tract from the bladder to the skin was lined by granular, grayish white tumor. The lower ureters were surrounded by neoplastic tissue, but were not dilated. The kidneys showed no metastases, and were not hydronephrotic. The rectum was extensively invaded by tumor, but the mucosa was essentially intact. The thyroid

gland was greatly and irregularly enlarged, in part rubbery, in part soft and fleshy. Lymph nodes were not grossly enlarged nor involved by tumor. There was a large duodenal ulcer, 3 by 4 cm., situated 2 cm. distal to the pylorus.

Microscopic Examination. The surgical specimen showed a well marked, characteristic, nodular prostatic hyperplasia. Many portions were extensively invaded by thickly clustered masses of discrete dark-staining cells. A scanty delicate stroma coursed between individual cells and small clusters or groups of tumor cells. Nuclei were uniformly dark-staining and chromatin-rich. Cytoplasm was generally polyhedral and amphophilic. Large, pale histiocytes, frequently with phagocytized material, were seen occasionally. In some zones the infiltration surrounded persisting prostatic acini, but no transitional forms were observed between glandular epithelium and tumor. In other parts there were aggregates of cells closely resembling normal lymphocytes.

In the necropsy tissues there was some variation from site to site in the degree of differentiation of the neoplasm. In some areas small lymphocyte-like cells predominated, while in others the cells appeared more primitive (Fig. 5). Most zones showed a mixture of types.

The thyroid gland was extensively invaded by quite well differentiated cells. In the lung, tumor replaced lung parenchyma beneath the pleura, and elsewhere surrounded bronchi and small blood vessels, infiltrating interstitially. Large histiocytes laden with phagocytized material, not appearing neoplastic in any way, were abundant in the midst of the tumor elements. In the liver, a discrete metastasis replaced parenchyma and infiltrated the portal spaces at its margin. The pancreas was extensively infiltrated by mature and primitive lymphoid cells with considerable compression atrophy of pancreatic acini. The omentum and mesentery exhibited very diffuse permeation. The cells were diffusely scattered, never exhibiting follicular arrangement. Very wide variations in cell forms were noted, ranging from angular, polyhedral, dark cells to normal lymphocytes and histiocytes. Areas of necrosis were present in the omentum.

The tumor mass in the pelvis was substantially similar to the tumor elsewhere. The rectum was infiltrated in all layers except the mucosa by discrete cells of very wide range of maturity. The duodenal ulcer showed very extensive infiltration of the base (Fig. 6). The wide morphologic range was prominent here, and in addition many eosinophils were seen.

Lymph nodes in this case revealed preserved architecture, with distinct sinusoids and cellular lymph cords. Primary follicles were present but no germinal centers. In the sinusoids were abundant, large, pale

cells of endothelial type with many lymphocytes. There was moderate variation in degree of maturity of the lymphocytes in the lymph cords, but the general appearance was that of a mildly hyperplastic node. The capsule and immediately surrounding adipose tissue were occasionally infiltrated by regular, mature-appearing, lymphoid cells.

The spleen revealed no gross evidence of tumor. Microscopically, the structure was well preserved, but occasional cells, isolated or in small clusters within the sinusoids, resembled primitive cells of undetermined type. Numerous cells indistinguishable from eosinophilic myelocytes suggested that the undifferentiated cells were myeloid elements, of the type frequently seen in the spleen. Bone marrow sections showed cellular marrow, with no evidence of neoplastic infiltration.

A single microscopic focus of immature lymphoid cells was found in the medulla of one kidney.

The final pathologic diagnoses were: chronic peptic ulcer of duodenum, with fresh hemorrhage; lymphoblastoma of the prostate, with metastases to periprostatic and pelvic tissues, rectum, bladder, mesentery, omentum, lungs, liver, thyroid gland, kidney, and to base of duodenal ulcer. Subsidiary diagnoses were: healed adhesive pericarditis (cause undetermined); emphysema; fibrous pleural adhesions.

Case 3

The patient was a male, 76 years old, who entered with dysuria, hematuria, and suprapubic pain of about 3 weeks' duration. Physical examination was non-contributory. Laboratory data showed urine loaded with red blood cells and numerous white blood cells, 2 plus albumin, non-protein nitrogen elevated to 48 mg. per cent, and urine culture positive for *Aerobacter aerogenes*. The red blood cell count was 4.0 million; white blood cells, 12,800; 80 per cent polymorphonuclear cells. Serologic examination was negative. Acid phosphatase, 0.5 units. Cystoscopic examination revealed a large, necrotic, fungating, papillary tumor involving most of the right half of the bladder. A hemi-resection of the bladder was performed, and a pathologic diagnosis of papillary transitional cell carcinoma of the bladder was made. A small, similar, papillomatous nodule was removed from the urethral orifice.

The patient did fairly well. But when the catheters were removed, difficulty in voiding resulted. Six weeks after the bladder operation a transurethral prostatic resection was required. The pathologic diagnosis was carcinoma of the prostate, highly undifferentiated, with the notation that the tumor appeared completely undifferentiated, and morphologically reminiscent of a reticulum cell sarcoma. The patient was discharged in good condition, urinating 4 to 6 oz. every 3 to 4 hours. He was re-admitted 12 days later because of retention. A fistula was present in the suprapubic wound. The bladder was drained by an indwelling Foley catheter. Initial laboratory work showed red blood cells, 3.6 million; white blood cells, 9,600; differential, 83 per cent polymorphonuclear cells; hemoglobin, 9.8 gm. His temperature ranged from 98° to 100° F. He complained of severe pain in the upper abdomen, nausea, and epigastric tenderness. Amylase was 4 units. Roentgenograms showed non-visualization of the gallbladder, while a gastro-intestinal series revealed a hiatus hernia with deformity of the duodenal bulb consistent with an active duodenal ulcer. Traces of occult blood were present in the stools on two of three examinations. He was kept

on a modified ulcer diet but suffered from severe anorexia. The total serum protein was 5.1 gm. per cent. A medical consultant believed the condition was primarily disseminated carcinomatosis, with no indication for surgical interference. He expired 3 weeks after admission, and about 5 months after onset of symptoms.

Necropsy Findings

Necropsy disclosed the following significant features: There was a well healed suprapubic scar. No relevant abnormality was present in the thorax. Adhesions were present in the region of the gallbladder. The colon was greatly distended. Tumor was visible grossly only in the pelvis. The prostate was replaced by a mass, 12 by 10 cm., grayish white and encephaloid in consistency. The tumor involved the wall of the rectum over a 0.7 cm. area, causing marked stenosis. The seminal vesicles were replaced by tumor. The bladder was markedly decreased in size. There was a single erosion in its fundus, but only the floor was involved by the pelvic tumor. No papillary tumor was observed. No duodenal ulcer was identified.

The first surgical specimen, resected from the bladder, contained an entirely characteristic, papillary, transitional cell carcinoma. At the base of the tumor and in the stroma of the papillae were abundant, mature, typical lymphocytes. On review of the slides small zones of closely crowded, polyhedral, dark-staining cells were observed, interpreted now as neoplastic, although their significance was originally overlooked. In the second surgical specimen, from the prostatic resection, there were areas of dense cellularity, the individual cells having small, dark nuclei, and irregular scanty cytoplasm, in general similar to the cells in case 1.

The necropsy tissues resembled those of case 2. The infiltrating cells were in large part indistinguishable from normal lymphocytes, but intermingled with the relatively mature cells were more primitive forms, judging by larger size, more irregular contours, alterations in nuclear configuration, chromatin pattern, and staining reactions, and other commonly accepted signs of lesser differentiation. Large, pale, phagocytic cells resembling normal histiocytes were seen occasionally but less frequently than in the other cases.

The pelvic mass obliterated all prostatic architecture, invaded the rectum, the floor of the bladder, and the contiguous soft tissues. The ulcer in the fundus of the bladder was non-specific, and no tumor cells could be identified. Relatively few distant metastases were present. A small nodule in the abdominal wall, in relation to the scar, was composed of poorly differentiated cells which nevertheless were discrete and not syncytial. A few microscopic foci of similar tumor cells were found in the wall of the gallbladder, which was the seat of mild chronic

cholecystitis with lithiasis. Although the pancreas was grossly normal, the peripancreatic adipose tissue contained abundant lymphocytoid cells, generally quite mature. No other distant metastases were noted. The distal ureters were involved, with moderate hydro-ureter. Moderately severe pyelonephritis was present on the left, but no tumor was present in the kidneys.

The lymph nodes were small, with excellently preserved architecture. The lymph cords were only sparsely cellular; follicular formation was not prominent, and the sinusoids were very distinct and not unduly cellular. Occasional discrete, immature-appearing cells were seen in the lymph cords and sinusoids. The capsules and perinodal adipose tissue were free of tumor. The bone marrow was moderately hyperplastic. There was no trace of neoplastic infiltration. The spleen, which weighed 200 gm., was congested and contained very abundant, mature polymorphonuclear leukocytes and some plasma cells. Neoplastic elements could not be identified. Abundant polymorphonuclear cells were present in the sinusoids of the liver, and to a lesser degree in the portal spaces, but no neoplastic cells were observed.

The final pathologic diagnoses were: scar of recent operation (segmental resection of bladder for transitional cell carcinoma); acute cystitis with focal ulceration; lymphosarcoma of prostate, with metastases to prostatic urethra, rectum, seminal vesicles, bladder, ureters, retroperitoneal fat, gallbladder, and abdominal scar; stenosis of ureters, with bilateral hydro-ureter, moderate left hydronephrosis, left acute ascending suppurative pyelonephritis; acute splenitis; stenosis of rectum, with marked dilatation of colon; slight ascites; marginal atelectasis; pulmonary edema. Subsidiary diagnoses were: severe generalized arteriosclerosis; focal fibrosis of myocardium; fibrous pleural adhesions; chronic cholecystitis with cholelithiasis.

This case is of special interest as exhibiting two primary malignant tumors, each arising in the genito-urinary tract.

Discussion

I

A problem which bothers second year medical students and recurs periodically to plague mature pathologists is the difficulty in distinguishing undifferentiated carcinoma from sarcoma. It is of interest that, in the 3 cases described, the initial diagnosis on the surgical specimens was undifferentiated carcinoma. Only after thorough necropsy study were we willing to make the definitive diagnosis of lymphoma, although that possibility was considered, as mentioned previously, in notes qualifying the pathologic reports.

There is no single morphologic criterion for distinguishing carcinoma from sarcoma, and rigid rules cannot be laid down. Certainly the reticulin pattern is a most unsatisfactory reed upon which to lean. Since specific standards are virtually never given in the literature, we wish to stress the following guiding points which have influenced our decisions. When cells are sufficiently undifferentiated their nature can be told only "by the company they keep." That is, when, among undifferentiated cells, some by transitional stages appear to form acini or sheets, or to cohere in the manner of epithelium, the undifferentiated cells are considered to be epithelial in nature. On the other hand, when undifferentiated cells apparently exhibit transitions into recognizable lymphoid cells, we think of the primitive forms as constituting some type of malignant lymphoma. In other words, we do not identify undifferentiated cells, but infer their nature from association with forms which we can identify. If, in a particular tumor, all cells are uniformly undifferentiated and no recognizable descendants are present, we must make our diagnosis by observing similarities to cells of other cases wherein descendants are recognizable. The necropsy data and photomicrographs furnish, in light of our criteria, the evidence to support the present diagnoses.

A further problem arises: How do we know if a tumor has originated in the prostate or has spread to the prostate by metastasis? The answer to this question is in part determined by the nature of the tumor and its distribution. For example, consider an undifferentiated tumor which is present only in the prostate, lymph nodes, and bone marrow. If the epithelial nature of this tumor could be established, the tumor must have arisen in the prostate. On the other hand, if the tumor is established to be a reticulum cell sarcoma, its origin remains in doubt, since it could have arisen from lymph nodes, bone marrow, or prostate. In such an instance other grounds must be utilized to decide between lymph node or prostate as the primary site of origin. Thus the problem of origin of a tumor may be bound up with the problem of the nature of the tumor.

In relation to the prostate, the problem of knowing whether a tumor arises in the prostate or has spread there by metastasis was discussed by Bettoni.[1] He declared that the prostate is virtually never involved by metastatic lesions, while the other organs affected in the case under discussion are common sites of metastases. Thus he concluded by exclusion that the prostate was the primary site. Bettoni begged the question and his theoretic contentions cannot be allowed. The fact that the prostate may be the site of metastases was recognized as long ago as 1869[2] and 1888.[3]

Assuming a case to be a lymphosarcoma, the primary origin in the prostate would be established if (1) there were no lymph node involvement, and if (2) the tumor were quite closely restricted to the prostate and immediately adjacent tissue. These conditions, if established by necropsy, would rule out origin from lymph node or other ordinary hematopoietic organs. Such conditions were met essentially by our case 3. The tumor was virtually restricted to the pelvis, with no involvement of lymph nodes and insignificant distant metastases in the gallbladder and pancreas. The structure of the tumor in the pelvis indicated invasion of the bladder from the prostate rather than the reverse.

When lymphosarcoma is present very widely in the body, the difficulty of determining the primary site increases in direct proportion to the extent of the tumor. In our case 2 the tumor was very widely disseminated. However, the absence of lymph node involvement ruled out the nodes as a site of origin, while similar considerations applied to bone marrow and spleen. That the diffuse lymphoid tissue in the mesentery or omentum formed the primary origin seems overwhelmingly improbable. The involvement of liver and lungs was characteristic of secondary or metastatic tumor. The thyroid gland and the rectum remained as possible sites, and could not be excluded definitely. In fact, lymphoblastoma of the thyroid gland and of the gastro-intestinal tract are well recognized entities. We rejected these sites, however, on the ground that symptoms were first referable to the prostate. Lymphoblastomas are not recognized as behaving like certain epithelial tumors such as lympho-epitheliomas, or some hypernephromas or bronchogenic carcinomas, wherein bulky metastases stem from very small primary lesions. We make explicit the postulate that in malignant lymphomas the first site of symptoms is most acceptable as the primary. In this connection it must be emphasized that the determination of the "first site" requires careful and detailed study, and not merely the casual observation of a small lump. However, there remained in our case 2 a small but reasonable doubt that the prostate actually was the site of origin of the tumor. We made our diagnosis on the basis of strong probability, drawn from correlation of clinical and pathologic findings.

Case 1 presented a different problem. In case 1 there was extensive lymph node involvement, together with a small nodule in the spleen. The splenic nodule was so small and so sharply limited that it can be dismissed as a primary source. But the involvement of the lymph nodes cannot be so easily dismissed. Our sole grounds for diagnosing the prostate as the primary site consisted of the clinical primacy of

prostatic symptoms, and, pathologically, of the enormous preponderance of the pelvic tumor which by history and physical examination seemed to begin in the prostate. This case may be contrasted with that of Taschiro [4] which we rejected as a primary lymphoma of the prostate on the grounds that, in his case, lymphoma was very widespread in the body and no symptoms pointed to prostatic involvement during life; while, at necropsy, the prostatic involvement was not disproportionate in comparison with other organs.

The nomenclature of lymphoid tumors is confused, and there is difference of opinion regarding the inter-relations of the various subdivisions. We believe that the term malignant lymphoma can be applied to all tumors which stem from primitive syncytial mesenchyma and which are developing toward the mature lymphocyte, but which may also give rise to other mesenchymal derivatives. Such tumors can be considered, by analogy, as a continuous line, with the most primitive form at one end, the most mature at the other. The line can be subdivided into any arbitrary number of subdivisions. Different observers would disagree on the suitable numbers of subdivisions, as well as on the exact position of the point marking off one subdivision from another. For greater accuracy in the analogy, the straight line should be construed as having diverging side-shoots, which sometimes form connections with other side-shoots. Certain of the lymphomas seem to differentiate into forms not found in the normal course of development of the lymphocyte. Such aberrant forms may become transformed into other types of lymphoma. We strongly endorse Custer and Bernhard's [5] viewpoint of the inter-relations and inter-penetrations of the lymphoma group.

In matters of nomenclature, we believe that the terms lymphosarcoma, malignant lymphoma, and lymphoblastoma should be used interchangeably to indicate any tumor of lymphoid character, regardless of the degree of differentiation. We particularly deplore the use of the term lymphosarcoma to indicate only the tumor composed of well differentiated mature lymphocytes. Stirling and Ash,[6] for instance, inveighed against the term reticulum cell lymphosarcoma as a hybrid, of which only one-half could be correct. They differentiated reticuloendothelial sarcoma from lymphosarcoma. In our terminology, lymphosarcoma, malignant lymphoma, and lymphoblastoma are used interchangeably as generic terms, with or without the specific or subdividing qualifications of lymphocytic type, reticulum cell type, stem-cell type, or mixed type. In accordance with our analogy, lymphoma and its synonyms designate the continuous line denoting all lymphoid tumors from the most primitive to the most mature. The qualifying terms

TABLE I

Malignant Lymphoma (Lymphoblastomas) of Prostate, Verified by Necropsy

Author	Year	Age	Duration from onset of symptoms	Pathologic findings
King and Cox Case 1	1951	60	10 months	Malignant lymphoma of prostate, reticulum cell type, with metastases to pre-aortic, mesenteric, and abdominal lymph nodes; spleen; ureters; seminal vesicles; periprostatic tissues
King and Cox Case 2	1951	76	6 weeks	Lymphoblastoma of prostate with metastases to periprostatic and pelvic tissues, rectum, and bladder; mesentery; lungs; liver; thyroid gland; kidney; base of duodenal ulcer
King and Cox Case 3	1951	76	5 months	Lymphosarcoma of prostate with metastases to rectum, bladder, seminal vesicles, and contiguous soft tissues; gallbladder; distal ureters; abdominal scar; retroperitoneal fat; prostatic urethra
Kirshbaum, Lagkin, and Culver[8]	1943	22	3 years	Reticulum cell sarcoma of prostate with metastases to lungs, bladder, liver, left adrenal, and periaortic nodes
Kaufmann and Wright[9]	1941	62	6 months	Lymphosarcoma of prostate with metastases to surrounding tissues, bladder, ureters, deferent ducts, kidney capsule, rectum, sigmoid, pancreas, periadrenal fat, mesentery, skin, lungs, pleura, abdominal lymph nodes
Rathbun and de Veer[10]	1938	52	7 months	Lymphosarcoma of prostate with metastases to rectum; lymphosarcoma of jejunum, regarded by authors as second independent tumor; tumor in lymph nodes around jejunum
Smith[11]	1937	44	6 months	Lymphosarcoma of prostate with metastases to bladder, pre-aortic, and perirenal lymph nodes only
Cole and Martin[12]	1934	56	12 months	Lymphosarcoma of prostate with metastases to pancreas and bladder neck only
Mason[13]	1933	41	265 days	Malignant lymphocytoma of prostate with metastases to seminal vesicles, bladder, kidneys, adrenals, pancreas, Glisson's capsule, and mesentery
Ferguson and Stewart[7]	1932	36	1 year	Reticulum cell sarcoma of prostate with metastases to pelvic, aortic, and inguinal nodes, kidneys, bladder, and seminal vesicles; leukemic infiltration of spleen and liver
†Costa[15]	1928	45	History inadequate	Small round cell sarcoma of prostate with metastases to pleura, heart, liver, gallbladder, pancreas (almost totally replaced), gastro-intestinal tract, lymph nodes, small nodule in spleen, nodules in kidney

†Costa[15]	1928	45	History inadequate	Small round cell sarcoma of prostate with metastases to pleura, heart, liver, gallbladder, pancreas (almost totally replaced), gastro-intestinal tract, lymph nodes, small nodule in spleen, nodules in kidney
Schuler[16]	1927	70	15 months	Small round cell sarcoma of prostate with metastases to the bladder, periaortic, and inguinal lymph nodes
Symmers[17]	1923	30	2½ months	Lymphosarcoma of prostate with metastases to bladder, psoas muscles, periaortic, and peripancreatic nodes, left kidney, and left adrenal
Conforti and Favento[18]	1907	45	4 months	Primitive lymphosarcoma of prostate with metastases to heart, ureters, and bladder
Proust and Vian[19]	1907	19	13 months	Sarcoma parviglobocellulare of prostate with metastases to kidneys and bladder
Coupland[20]	1877	29	5 months	Lymphosarcoma of prostate with metastases to bladder, pancreas, and right adrenal

* Patient died of pneumonia. Urinary symptoms present only 1 day before death.
† The history is inadequate in this case, but we accept this case on the basis of the pathologic description.

TABLE II

Malignant Lymphoma of Prostate, Diagnosed on Biopsy Material Alone

Author	Year	Age	Interval from onset of symptoms to death	Pathologic findings
Pease and McDonald[37] Case 1*	1947	57	Still living at time of report	Lymphosarcoma of prostate; palpable submental, cervical, inguinal, and axillary lymph nodes on physical examination
Pease and McDonald[37] Case 2*	1947	51	Still living at time of report	Lymphosarcoma of prostate; generalized lymphadenopathy on physical examination
Tenenbaum[38]	1942	65	18 months (no necropsy)	Lymphosarcoma of prostate and epididymis; metastasis to rectum, biopsy; generalized lymphadenopathy on physical examination
Counseller and Bedard[39]	1940	21	6 months (no necropsy)	Lymphosarcoma of prostate; enlarged lymph node in groin on physical examination
Dial[40]	1934	59	13 months (no necropsy)	Lymphosarcoma of prostate; metastasis to inguinal node, biopsy
Paul[41]	1929	14	10 months (no necropsy)	Lymphosarcoma of prostate; metastasis to perineal incision; lung metastases, radiographic
Quinby[42]	1920	41	4 months (no necropsy)	Lymphoblastoma of prostate

* These cases present very scanty clinical or morphologic data, but in all probability are acceptable.

indicate the points on the line. Since the position of the differentiating points will vary from one observer to another, so will the exact qualifying terms. It is important to identify a tumor as belonging to a particularly directed mesenchymal derivative; it is less important to identify exactly what stage of differentiation has been attained.

In our own series we use the term reticulum cell as indicative of a stage of development intermediate between the most primitive and the most mature forms of the lymphoid series. There is no implication of identity with the reticulum cell as found in the normal spleen, or bone marrow. We consider that the neoplastic cell in reticulum cell sarcoma still retains potentiality for differentiation into various mesenchymal derivatives, and yet has progressed beyond the primitive or stem-cell lymphoma. The lymphocytic type is most mature, and has lost the power of further differentiation.

In reference to lymphomas arising in the prostate, much concern has been expressed in the literature because of the virtual absence of mature lymphocytes in the normal prostate. Ferguson and Stewart,[7] among others, believed that their tumor arose from chronic lymphoid infiltration of old gonorrheal infection. We disagree sharply with the philosophy behind this point of view. If lymphoid infiltration on an inflammatory basis were a significant factor in the production of lymphosarcoma, we should expect lymphosarcoma of the cervix to be the most common tumor in women. The presence or absence of lymphoid tissue under normal or pathologic circumstances need have no bearing, we believe, on the rise of lymphomatous tumor. We believe that malignant lymphomas can arise in any organ which contains mesenchymal derivatives. Although inflammatory change is not a necessary precursor, under conditions of inflammation there may be a stimulation of pre-existing primitive cells of mesenchymal origin. Whether these are designated as mesenchymal cells, pericytes, retothelial cells, reticulum cells, or by other terms, is of no great moment. The particular designation depends upon where, on the histogenetic line, the observer wishes to divide one cell type from another. These primitive or undifferentiated cells are not ordinarily seen under normal circumstances, but their existence is inferred from the nature of inflammatory infiltrations. Such cells, if their existence in normal tissues is granted, can be stimulated to neoplastic proliferation through the unknown factors which provoke tumors. It is not necessary to postulate either normally existing mature lymphoid tissue nor lymphocytic infiltration of an inflammatory nature, before accepting the existence of a primary lymphoma in any given organ.

II

A careful review of the literature indicates that a definite group of cases can be segregated in accordance with the principle discussed above. In the literature several cases have been designated as lymphosarcoma, others as round cell sarcoma of the prostate. In Table I we have collected all of the cases which we believe satisfy pathologic and clinical criteria for malignant lymphoma of the prostate. The table includes only those instances which were verified by necropsy.

Concerning the data presented in Table I, a few words of comment are in order. In the case of Rathbun and de Veer [10] the patient developed a lymphosarcoma in the jejunum. This second tumor was discovered 3 weeks after his admission for urinary retention. The authors considered the tumors in the prostate and jejunum independent and coincidental, although the microscopic pictures were said to be identical. We see no adequate reason for not accepting the jejunal tumor as metastatic. This is especially true inasmuch as the prostatic symptoms antedated the earliest jejunal symptoms by 6 months. It is of interest that Ewing confirmed the diagnosis on the prostatic tissues.

Wassiljeff's [14] case presented the unusual feature of a total lack of urinary symptoms until 1 day before death. The patient was admitted for an inguinal hernia and apparently died of heart failure. We consider that this case satisfies our criteria on the basis of the size and location of the tumor. The spread was chiefly by local extension, to the bladder and seminal vesicles, and the only distant metastases were to the kidneys.

One of the current problems in the field of the lymphomas is the relation of leukemia to lymphosarcoma. In the case of Ferguson and Stewart,[7] for example, the authors described lymphosarcoma in the prostate but also mentioned a leukemic infiltration of the liver and spleen. However, they did not believe the case was one of leukemia, in spite of the very large size of the spleen and the portal infiltrations in the liver. We believe that there is no fundamental difference between lymphatic leukemia and lymphosarcoma. In the former there are significant numbers of neoplastic cells in the circulating blood, in the latter, none. But from the standpoint of histopathology there is no criterion, such as aggressiveness nor the location of lymphocytes in the tissue, which can differentiate one from the other. The problem at issue is whether the lymphomatous tumor took origin in the prostate. We accept the case of Ferguson and Stewart as of this type. Whether, having so arisen, the cells invade the blood stream in significant numbers, has, in our opinion, very little bearing on the nature of the tumor.

In this connection, cases of lymphatic leukemia of McCrea [21] and Jacobi, Panoff, and Herzlich [22] are not acceptable as sarcoma of the prostate. The gland may have been invaded, but there is no evidence that the neoplasm took origin at this site. The presence of lymphomatous cells in the prostate is not by itself adequate grounds for inclusion in this category of primary sarcoma.

In addition to the 17 cases which we have tabulated, cases of Bettoni [1] and Taschiro [4] were not accepted because there was no adequate evidence that the primary tumor was in the prostate. We believe that the primary disease was malignant lymphoma, but the small size of the prostatic tumor and the extensive involvement elsewhere in the body make the site of origin a matter of conjecture.

A case of E. Kaufmann,[23] although accepted by Ferguson and Stewart,[7] is rejected by others.[11,12,17] We agree with the latter group in considering the data presented as inadequate for inclusion in the present category. It is possible that true lymphosarcoma of the prostate may be present among other cases in which the data were inadequate to determine the true nature of the tumor or its exact site of origin. In this category we have placed the 2 cases of Socin,[24] and those of Scheer,[25] Schöppler,[26] Spitzer,[27] Cahen,[28] Rose,[29] Wharton,[30] Clegg,[31] and Jolly [2]; as well as 4 cases [32-35] described in inaugural dissertations, and not available in the original; and a further case by Maraini [36] which we have been unable to consult.

In the literature, we have found 20 cases of lymphoma or round cell sarcoma of the prostate which had been diagnosed by biopsy alone. The absence of necropsy findings makes evaluation much more difficult. In Table II we have tabulated 7 cases which we believe to be true primary lymphomas of the prostate.

Besides the data in Table II, Bumpus [43] presented 2 cases of alleged lymphosarcoma of the prostate in 1925. The first patient was a 28-year-old white male with a greatly enlarged prostate and bilateral inguinal lymphadenopathy. The tumor did not respond to radiation, and he expired 6 months after the onset of symptoms. The second case was a 50-year-old white male with a bilaterally enlarged, hard prostate. There was excellent response to radiation therapy and the patient was alive 2 years later. These 2 cases may indeed be true lymphosarcoma but, because of the absolute lack of morphologic description, we are not justified in including them in our table.

In 1925, Culver [44] published a case of large round cell sarcoma of the prostate in a 12-year-old boy. This tumor appears consistent with a lymphosarcoma. However, in this instance, there was a second tumor in the jaw which was reported to be morphologically identical to the

prostatic tumor. This second tumor, together with roentgenologic evidence of enlarged mediastinal lymph nodes, casts strong doubt upon the primary origin of the tumor in the prostate. This is especially true in the absence of necropsy.

In 10 other cases [45-53] diagnosed by biopsy as round cell sarcoma, the available data presented do not warrant inclusion as primary lymphoma of the prostate, in our opinion.

Of all our accepted cases of prostatic lymphomas, in both the necropsy and surgical groups, 7 patients received x-ray therapy. In 2 of these the patients received no benefit whatever. The improvement in the other 5 cases was transitory. The subjective symptoms reappeared and the tumor mass rapidly enlarged. The duration of the remission of symptoms varied from 1 week to 17 months. Among the necropsy and surgical cases only one patient survived more than 15 months from the onset of symptoms. The average duration from the onset of symptoms to death was 8 months. The exception was Kirshbaum's [8] patient who responded well to x-ray therapy and lived 3 years.

Summary

Three cases of lymphomatous tumors arising in the prostate gland occurred in patients whose ages were 60, 76, and 76 years respectively. In 2 instances the tumor was substantially limited to the pelvic organs, but the third showed more distant metastases. In only one was there lymph node involvement. In all, the initial and primary clinical features were referable to the prostate gland.

One case was unique in having a papillary transitional cell carcinoma of the bladder in addition to the lymphomatous prostatic tumor.

Histologically, all neoplasms were composed of lymphocytes and lymphoid cells together with more primitive undifferentiated cells which are interpreted as precursors of lymphoid elements and not as epithelial in nature.

All lymphomatous tumors are inter-related and subdivisions in nomenclature are essentially artificial. Malignant lymphoma, lymphoblastoma, and lymphosarcoma are regarded as interchangeable terms.

From a critical analysis of the literature, 17 necropsied cases, including those presented herein, are considered to be acceptable examples of primary lymphosarcoma of the prostate. In 7 additional cases the diagnosis, made on biopsy material alone, is considered as probable.

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DESCRIPTION OF PLATES

PLATE 124

FIGS. 1 and 2. Case 1. Prostatic tumor, from necropsy tissue. The moderately pleomorphic character of the cells is well shown, as is the delicate reticular stroma and its relation to the cells. Hematoxylin and eosin stain. × 600.

FIG. 3. Case 1. Metastasis to ureter. The dark cells are similar to those shown under higher power in Figures 1 and 2. There are several large, pale, mature histiocytes which are actively phagocytic. Hematoxylin and eosin stain. × 300.

FIG. 4. Case 1. Lymph node. There are numerous bizarre tumor giant cells, together with many histiocytes of non-neoplastic appearance. The giant cells are considered a consequence of radiation therapy. Hematoxylin and eosin stain. × 200.

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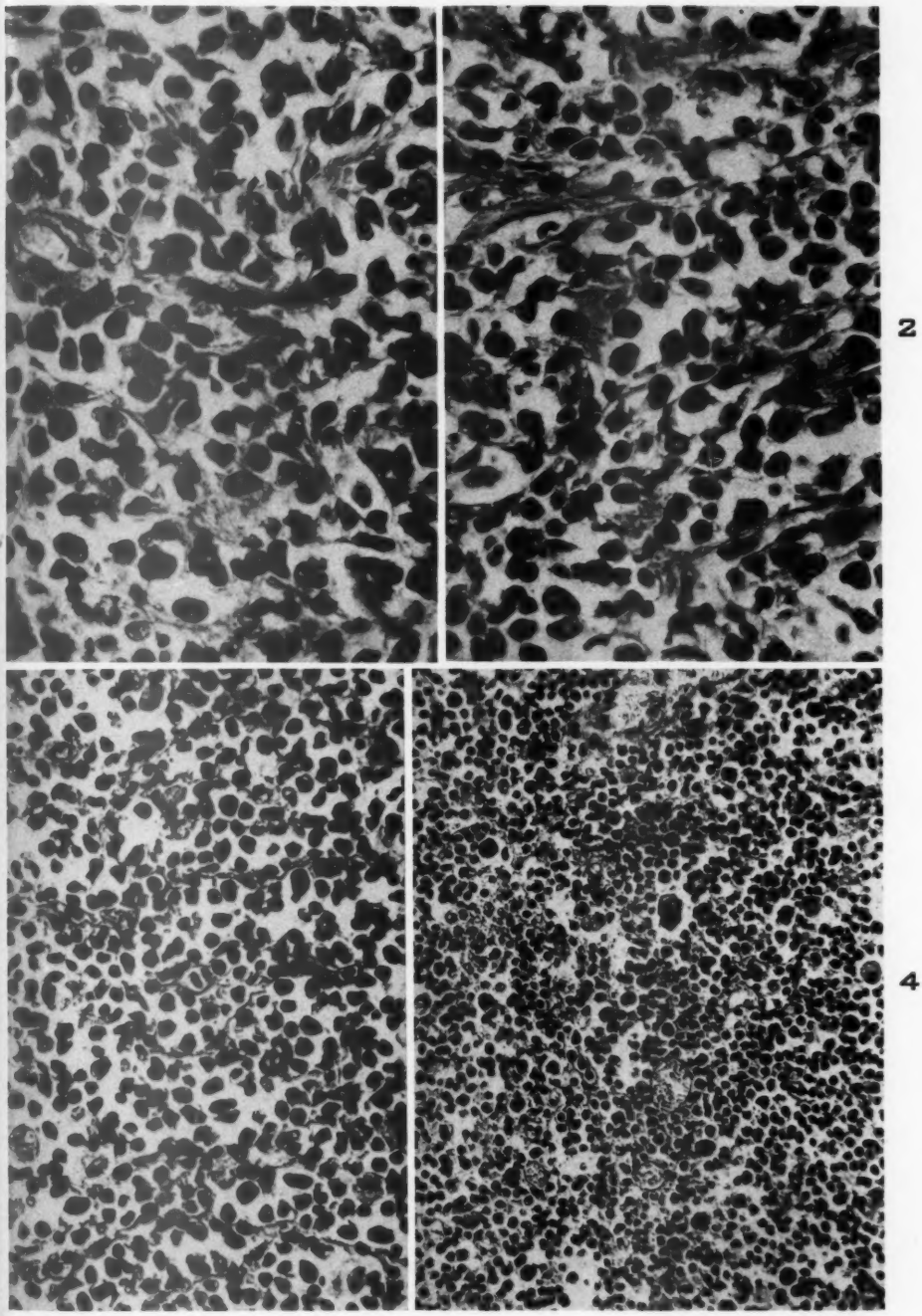


King and Cox Lymphosarcoma of the Prostate

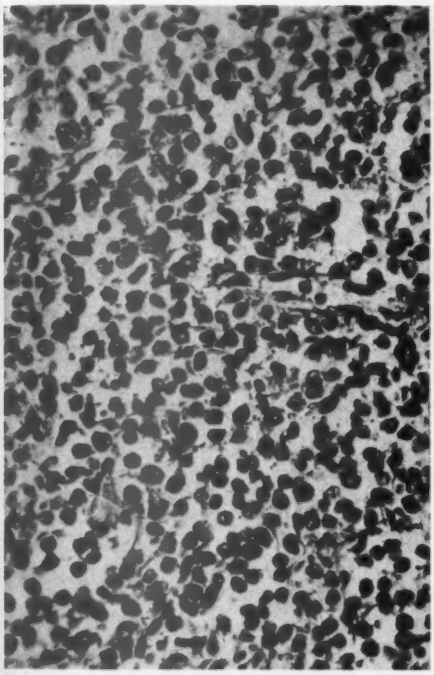
PLATE 125

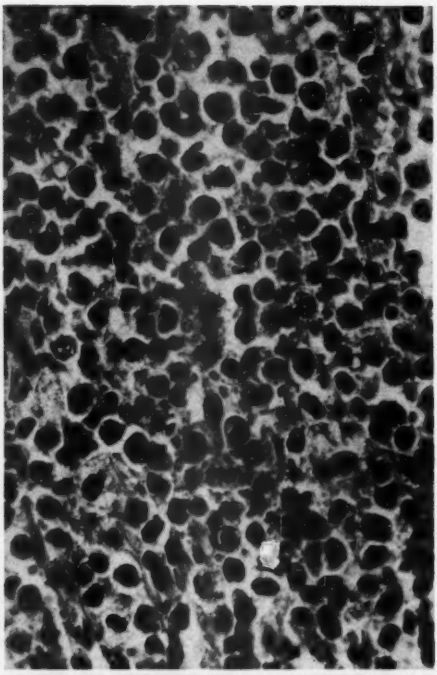
FIG. 5. Case 2. From edge of operative defect in prostate gland. Necropsy tissue. The cells are moderately variable in structure, ranging from small, dark cells comparable to mature lymphocytes to larger, more immature forms. Stroma is scanty. Hematoxylin and eosin stain. × 200.

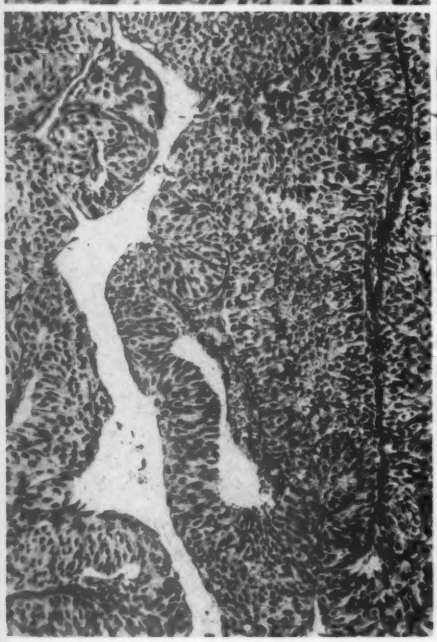
FIG. 6. Case 2. From the margin of the duodenal ulcer. The cells appear relatively primitive, with numerous mitotic figures. Some cells suggest differentiation towards histiocytoid forms. Hematoxylin and eosin stain. × 400.

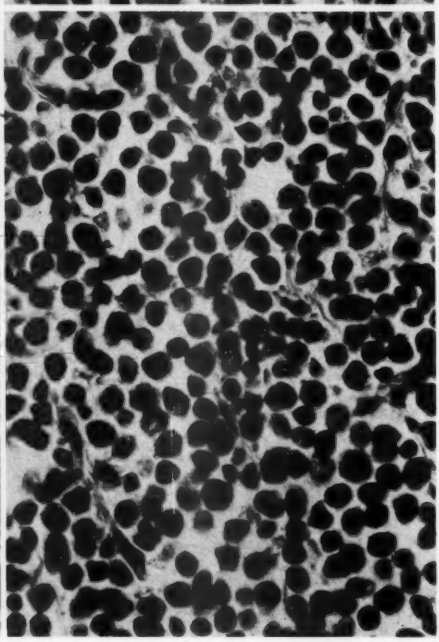
FIG. 7. Case 3. Papillary transitional cell carcinoma of the bladder, removed at first operation. Hematoxylin and eosin stain. × 115.

FIG. 8. Case 3. Prostate gland, necropsy tissue. The cells are large, dark-staining, of primitive appearance, and fundamentally similar to those in Figure 6. Hematoxylin and eosin stain. × 400.









King and Cox

Lymphosarcoma of the Prostate

THE NATURE OF DIFFUSE INTIMAL THICKENING OF ARTERIES *

SIGMUND L. WILENS, M.D.

(From the Departments of Pathology, Bellevue Hospital and New York University, College of Medicine, New York, N.Y.)

Atherosclerosis is generally thought to involve only isolated portions of the arterial intima. Much of the investigation of this disease has been influenced by this concept. Various agents that injure the arterial wall only at certain points, or intrinsic structural changes limited to these susceptible areas, have been sought to explain the localized nature of the process. The focal character of the lesions has been used also as a cogent argument against the hypothesis that a generalized disturbance, such as a dysfunction of lipid metabolism, is primarily concerned in the pathogenesis of this process.

Evidence has been presented,[1-3] however, that lipid-containing blood plasma may gain entrance into the arterial intima in a uniformly dispersed state and that the focal nature of the lesions may result from the subsequent redisposition and pooling of retained lipids within the intima. If this explanation is valid, one would expect to find diffuse changes throughout the arterial intima that can be attributed to such a uniform permeation.

At birth the intima of the aorta consists merely of a single layer of endothelium superimposed directly on its own reticular basement membrane or on that of the innermost elastic lamella of the media. The infantile intima is scarcely a true layer; it is merely a surface membrane. Early in life, however, the aortic intima acquires a subendothelial fibrous zone of rather uniform width and becomes separated from the media by a narrow zone of disorganized smooth muscle and elastic tissue that is commonly referred to as the musculo-elastic layer. This change occurs with such regularity and uniformity throughout the vessel that it was regarded by Jores,[4] and subsequently by most authors, as a normal developmental process. It was once considered to be related to arteriosclerosis. Mönckeberg [5] has summarized the various views that have been expressed concerning its significance. Recently, Dock [6] has considered fibrous intimal thickening of the coronary arteries to be an important precursor to the development of atherosclerosis. Currently, however, most authors do not regard diffuse intimal thickening as a part of the atherosclerotic process. Diffuse intimal thickening may be found without obvious demonstrable lipid deposition. It occurs early in life long before any evidence of atherosclerosis

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can be found, and also in the absence of visible intimal lipid streaks. There are, nevertheless, a number of circumstances that make this interpretation seem doubtful and suggest that this intimal change may be an early but consistent and integral part of the atherosclerotic process. The purpose of this report is to present the evidence on which this view is based.

The Distribution of Diffuse Intimal Thickening of Arteries

Diffuse intimal thickening does not occur in all arteries. Analysis of the distribution of this change reveals that it follows closely that of atherosclerosis. It is generally absent or minimal in the main pulmonary artery even in advanced age groups (Fig. 1), unless there has been prolonged pulmonary hypertension. In the latter case both diffuse fibrous thickening and discrete lipid-containing plaques often make their appearance. Likewise, in the aorta the intima does not have the same thickness at all points but tends to have greater depth in those areas that are prone to develop lipid deposits or discrete plaques. This can be verified readily by examining the aortas of young persons without atheromatous plaques. Sections from the abdominal aorta regularly reveal a thicker intima than those from the ascending thoracic segment of the same vessel (Figs. 3 and 4). Sections from the dorsal portion of the descending thoracic aorta usually have a thicker intima than those from the ventral wall at the same level (Figs. 5 and 6). These findings indicate that diffuse intimal thickening is generally more pronounced in those areas that are susceptible to early plaque formation and that this difference can be demonstrated before the plaques have formed.

Similarly, in arteries other than the aorta there are differences in the degree of intimal thickening. In general, arteries that are commonly the seat of atherosclerotic change are those that at an early age show relatively pronounced, diffuse, fibrous, intimal thickening. For example, the iliac and femoral arteries show this change more conspicuously than the carotid or innominate arteries from the same person (Figs. 7 and 8). Sappington and Horneff [7] found that intimal fibrous thickening was much more pronounced in the coronary arteries than in tibial or radial arteries from the same person. There was slightly more thickening in the tibial than in the radial arteries. Atherosclerosis occurs with about the same order of severity in these three vessels.

Factors Influencing the Degree of Diffuse Intimal Thickening

Additional findings that indicate that diffuse intimal fibrosis may be intimately related to the general process of atherosclerosis are provided

by an analysis of the factors that play a rôle in the development of these arterial changes. For this purpose the thickness of the intima of 626 routine paraffin-embedded sections of aorta was measured. These were taken at random from consecutive necropsies at Bellevue Hospital. Sections that did not include at least 1 cm. of flat, plaque-free intima were excluded. The thickness of the intima, in micra, at its narrowest point from the endothelium to the first medial elastic fiber visible in sections stained with hematoxylin and eosin, with the substage condenser shutter partly closed, was measured by means of an ocular micrometer.

These measurements are open to several objections and at best are only an approximation of the true intimal thickness. In many instances it is not possible to determine the exact point of junction with the media even in Weigert's elastic tissue stains. Secondly, since sections were taken from no constant part of the aorta, those from the ascending and anterior descending thoracic portions would tend to have a thinner intima than those taken elsewhere. It is likely that plaque-free sections from severely sclerotic aortas were frequently obtained from these areas. Finally, the aorta undergoes progressive enlargement with advancing age. The surface area of this vessel when freed from its *in situ* attachments is twice as great in a 60-year-old person as in one 20 years old. If this dilatation is accompanied by intimal stretching, it is obvious that the intima of older persons would tend to be thinner unless a compensatory overgrowth has occurred. Nevertheless, if the limitations of the method employed are taken into account, it is possible to obtain valuable information concerning the factors that regulate intimal thickness.

Although thickening of the intima begins at a very early age, long before the onset of atherosclerosis, it is like the latter process in that it progresses throughout life (Table I). It is unlike most developmental processes that become stabilized at maturity. The thickness of the intima does not increase greatly with each successive decade as measured in histologic sections. When, however, the degree of enlargement of the vessel that accompanies this thickening is considered, the actual amount of new intimal tissue formed independent of intimal plaques is impressive. For example, it can be estimated that there is approximately six times as much intimal tissue in a plaque-free aorta from a man 60 to 69 years old as in a man 20 to 29 years of age.

It may be noted (Table I) that the mean thickness of the aortic intima is appreciably greater in men than in women for every decade in which an adequate number of observations were made. In this respect diffuse intimal thickening resembles atherosclerotic plaque formation

which usually begins at an earlier age and progresses more markedly in men than in women. The sex difference shown in Table I is most pronounced in the first decade. This is due to the fact that several older children were included among the 17 boys, whereas 7 of the 11 girls were infants less than 2 months of age. But even among the in-

TABLE I

Influence of Age and Sex on Diffuse Intimal Thickening of the Aorta in Non-hypertensive Persons

Age	Male		Female	
	No.	Mean thickness of intima	No.	Mean thickness of intima
years		μ		μ
0–9	17	31	11	5
10–19	5	66	5	51
20–29	7	67	8	66
30–39	27	115	13	85
40–49	50	163	20	117
50–59	82	177	23	163
60–69	70	198	22	158
70–79	36	225	27	219
80 and over	8	208	9	236
Total	302	170*	138	142*

* Average by persons.

fants, the aortic intima of the male was noticeably thicker than that of the female. Similar findings were noted by Dock [6] in his study of infantile coronary arteries.

Diffuse intimal thickening, like atherosclerosis, is markedly accelerated in the presence of hypertension (Table II), particularly in the period from 30 to 60 years in men and 30 to 70 years in women. This observation is not surprising as it is well recognized that hypertension will lead to concentric intimal proliferation of small arteries and arterioles, a process that may be wholly analogous to diffuse intimal thickening of large arteries.

The series of 626 aortas includes 83 that came from emaciated persons. There is some evidence that emaciation may retard the development of atherosclerosis.[8] The 83 cases were scattered among the many subdivisions of age and sex, so that a direct comparison of intimal thickness by groups is not feasible. However, in 44 of the 83 (53 per cent), the intimal thickness was less than the mean thickness of the

various groups in which these aortas belonged. This would indicate that emaciation has little or no effect in retarding diffuse intimal thickening as measured in random histologic sections.

It has been claimed that both diabetes and obesity [8] may accelerate the development of atherosclerosis. Among the 31 diabetic persons

TABLE II

Influence of Hypertension on Diffuse Intimal Thickening of the Aorta

Age	Male Hypertension or cardiac hypertrophy				Female Hypertension or cardiac hypertrophy			
	Without		With		Without		With	
	No.	Mean intimal thickness	No.	Mean intimal thickness	No.	Mean intimal thickness	No.	Mean intimal thickness
years		μ		μ		μ		μ
30–39	27	115	5	159	13	85	2	210
40–49	50	163	9	246	20	117	8	218
50–59	82	177	23	203	23	163	10	257
60–69	70	198	40	217	22	158	22	242
70–79	36	225	31	248	27	219	14	212
80 and over	8	208	13	233	9	236	9	249
Total	273	181*	121	224*	114	164*	65	236*

* Average by persons.

included in this series, the intima was thicker than that of the mean of their respective age and sex groups in only 11 (35 per cent). Among 23 obese persons the intimal thickness exceeded the corresponding mean in only 9 (39 per cent). This would suggest that, if anything, diabetes and obesity retard diffuse intimal thickening. These unexpected findings probably are explained by the fact that in both these groups the plaque-free areas from which the sections were taken most often, were limited to regions in which the intima remains relatively thin. Similarly, in emaciated persons with relatively few atherosclerotic plaques a higher proportion of the sections probably were taken from the posterior wall of the descending thoracic and abdominal portions of the vessel.

Diffuse Intimal Thickening in Laboratory Animals

Diffuse intimal thickening has not been described as a natural developmental phenomenon in the aortas of aged animals, although localized areas of intimal fibrosis sometimes are found.[9] Rats that have died of spontaneous causes after a life span of about 3 years

regularly reveal complete absence of both aortic atherosclerosis and diffuse intimal fibrosis.[10] The short life span of most animals makes it difficult to compare the development of arterial disease in them and in man, but diffuse intimal thickening becomes noticeable in man in the first few years of life. It would seem unlikely that a natural structural peculiarity in arteries would be found in only one species. A more plausible explanation for this unusual finding in man is that it represents an abnormal change that in some fashion is related to the later occurrence of atherosclerosis.

Further support for this concept can be obtained by reviewing the findings in experimental atherosclerosis of rabbits produced by cholesterol feeding. Anitschkow [11] noted that when cholesterol feeding was stopped for a sufficiently long period before sacrifice, areas of diffuse fibrous intimal thickening devoid of lipid could be found in the rabbit's aorta. He explained this finding by assuming that these areas had previously contained lipid and that the latter had been resorbed but that enough connective tissue proliferation had taken place to leave the intima permanently thickened. The patchy nature of experimental atherosclerosis and the close resemblance of this lesion to the spontaneous lipid streaks found early in life in man have been emphasized in many reports. Histologic study of the aortas of rabbits that have been fed relatively small amounts of cholesterol for protracted periods of time reveals that the process is often quite diffuse and continuous over the entire surface (Fig. 10). Resorption of lipid from such an aorta would leave a somewhat uniformly thickened fibrous intima.

That such a sequence of events may be involved in the development of diffuse aortic intimal thickening in man is further suggested by the fact that lipid staining of aortas in which there is no grossly visible lipid often reveals lipid-impregnated fibers and scattered fat-laden cells throughout an area of apparently non-lipid-containing fibrous thickening. The fact that this intimal change occurs early in life does not exclude the possibility that transient periods of lipid deposition are involved in its development since lipid streaks have been noted in infancy.

Discussion

It can be seen from the foregoing observations that a considerable body of evidence can be compiled to support the view that diffuse intimal fibrosis is in some fashion related to the general process of atherosclerosis. It may, in fact, be a precursor to atherosclerosis. The constant association of the two raises the possibility that atherosclerosis

will not develop unless preceded by diffuse intimal thickening. However, atherosclerosis frequently fails to develop after diffuse thickening is pronounced, so that it is obvious that this latter change alone cannot be accountable for the subsequent development of atherosclerotic plaques. The presence of diffuse intimal fibrous thickening in persons of advanced age who have failed to develop discrete plaques is one of the main reasons why these two types of change have been considered to be mutually independent.

As already indicated, minute deposits of lipid sometimes are demonstrable with Sudan staining throughout diffusely thickened fibrous intimas. This observation, together with the analogies provided by the "healing" process of experimental rabbit atherosclerosis, suggests that this lesion results from the constant passage of lipid-containing fluid through the surface endothelium from the blood with temporary but repeated periods of stagnation within the subendothelial zone. Anitschkow [11] believed that the filtration of fluid in this fashion was physiologic and that lipid deposits would result if the lipid content of the transudate was abnormally high.

Anitschkow's basic concept [11] has been supported and enlarged upon in subsequent investigations.[1-3] It has been suggested that: (1) All areas of the intimal surface are uniformly permeable to fluid filtration; (2) at arterial pressures the endothelium is permeable to all non-corpuscular elements in the blood and not semipermeable as it is at capillary pressures; (3) under ordinary circumstances the passage of fluid through the intima is in a state of equilibrium, *i.e.*, its lipid content can be resorbed as rapidly as it enters, but that under abnormal conditions this equilibrium may be disturbed so that lipid material accumulates between the endothelium and the subjacent elastica; (4) pooling of lipid deposits first into streaks and later into discrete mounds is due to the mechanical effects of arterial pulsation on the lipid after its initial deposition in a dispersed state; (5) this intramural conglomeration is further accentuated during periods of active resorption of lipid when this material can be broken down and removed faster than new lipid is being added, since the lipid still in a dispersed state is more readily attacked than that which has already become pooled; (6) hypertension promotes the development of atherosclerosis by increasing the rate of transintimal fluid filtration; (7) hyperlipemia accomplishes the same end by enriching the lipid content of the intimal transudate; (8) different degrees of involvement of the various arteries in the same person may be attributed, at least in part, to differences in

arterial blood pressure in various parts of the body in upright positions.[6,3]

Viewed in the light of these concepts, the process by which the arterial intima becomes transformed from a simple endothelial cell membrane into a fibrous layer with considerable depth becomes apparent. It is apparent also why this transformation does not occur in portions of the vascular system where atherosclerotic plaques do not develop and why it is not found in species that are resistant to spontaneous atherosclerosis. Furthermore, it is easy to understand why even the simple fibrous thickening that precedes plaque formation tends to be more pronounced in areas that are likely to develop such plaques at a later period and why some of the factors that are believed to accelerate the development of atherosclerosis also lead to a more pronounced degree of diffuse intimal thickening.

Summary

Diffuse intimal thickening is more pronounced in arteries that commonly develop atherosclerosis than in those that are infrequently involved. It is present to a more marked degree in portions of the aorta that develop atherosclerotic plaques frequently than in those that seldom do so. Diffuse intimal thickening of the aorta increases progressively throughout life. It is more pronounced in men than in women. It is increased in the presence of hypertension. Diffuse intimal thickening of the aorta does not occur in species that do not develop atherosclerosis spontaneously. When cholesterol-fed rabbits are restored to a normal diet for protracted periods before sacrifice, fibrous intimal thickening of the aorta, analogous to that of man, is found. Lipid stains on the diffusely thickened aortic intima often reveal small deposits of lipid.

These observations suggest that the diffuse overgrowth of intimal tissue that occurs consistently early in life is not a natural developmental phenomenon but is related to the same mechanism that eventually leads to the formation of atherosclerotic plaques. This interpretation suggests that atherosclerosis is not a process that is limited to scattered areas of the arterial intima but is one that involves the entire intima.

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[*Illustrations follow*]

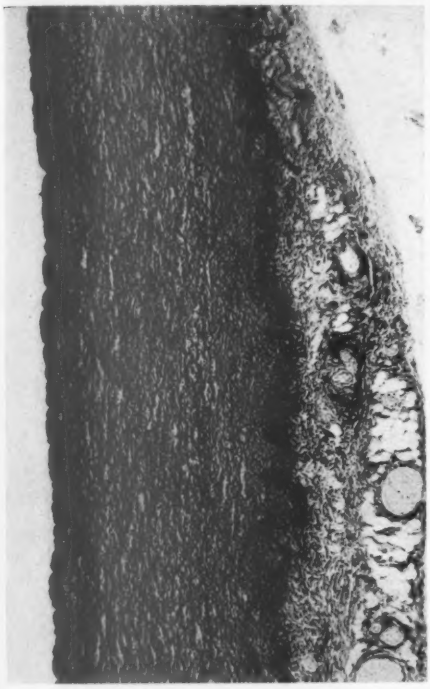
DESCRIPTION OF PLATES

All sections were stained with Weigert's elastic tissue and van Gieson's connective tissue stains. In Plates 126 and 127 the intima is at the left in each figure.

PLATE 126

FIGS. 1 and 2. Sections of pulmonary artery (Fig. 1) and ascending aorta (Fig. 2) at the same magnification (× 25) from a man, 48 years old. The intima of the pulmonary artery is as delicate as that of a newborn infant, while that of the aorta shows a slight but uniform fibrous thickening.

FIGS. 3 and 4. Sections of ascending (Fig. 3) and abdominal aorta (Fig. 4) at the same magnification (× 100) from a man, 25 years old. The intima of the ascending portion shows almost no thickening. That of the abdominal portion appears as a definite fibrous layer in which the innermost elastic fiber is separated from the underlying lamella to form an intermediate "musculo-elastic" zone.



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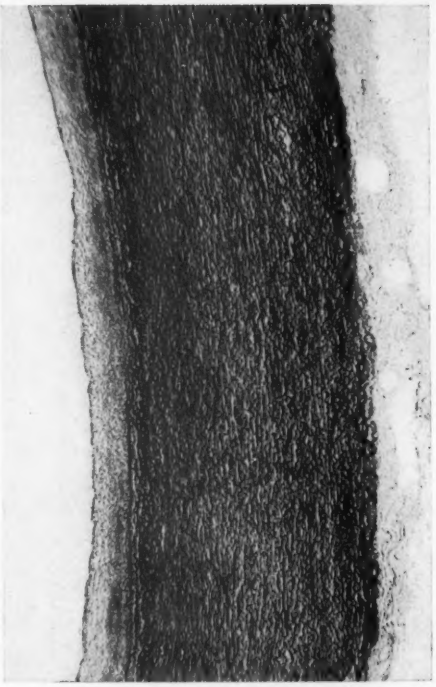

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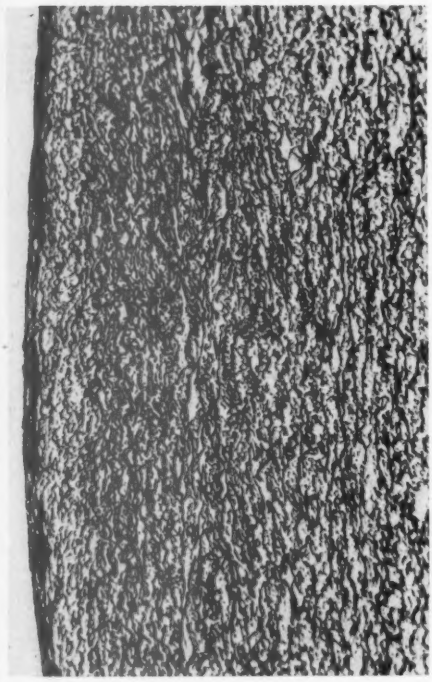

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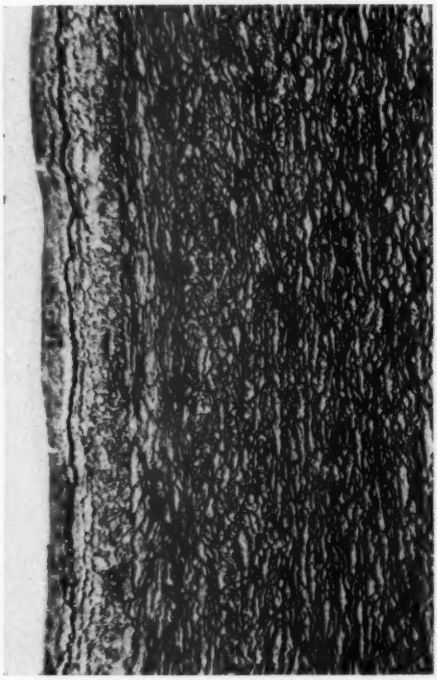


4

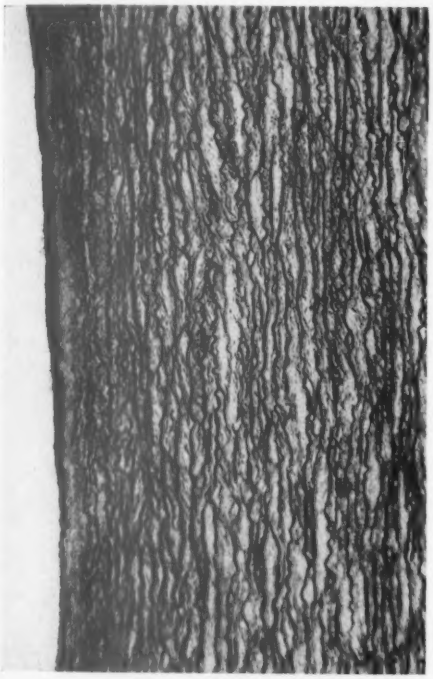
Wilens

Intimal Thickening of Arteries

PLATE 127

FIGS. 5 and 6. Sections of ventral (Fig. 5) and dorsal portions of the wall of the (Fig. 6) descending thoracic aorta at the same level and at the same magnification (× 100) from a man, 39 years old. The intima of the dorsal portion is three to four times as thick as that of the ventral portion.

FIGS. 7 and 8. Sections of common carotid (Fig. 7) and common iliac (Fig. 8) arteries at the same magnification from a man 52 years old. The intima of the iliac artery is several times as thick as that of the carotid artery.



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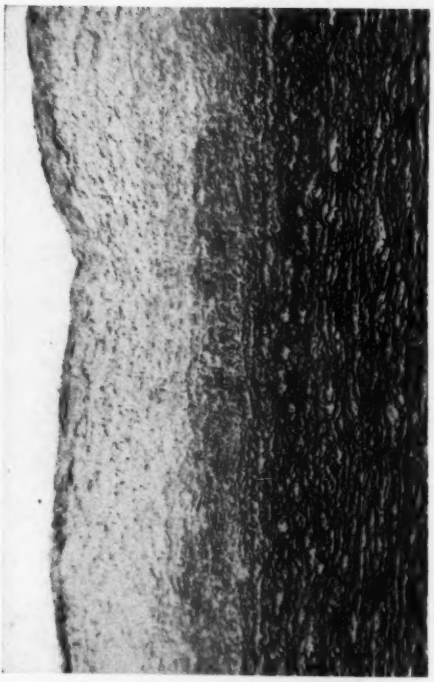

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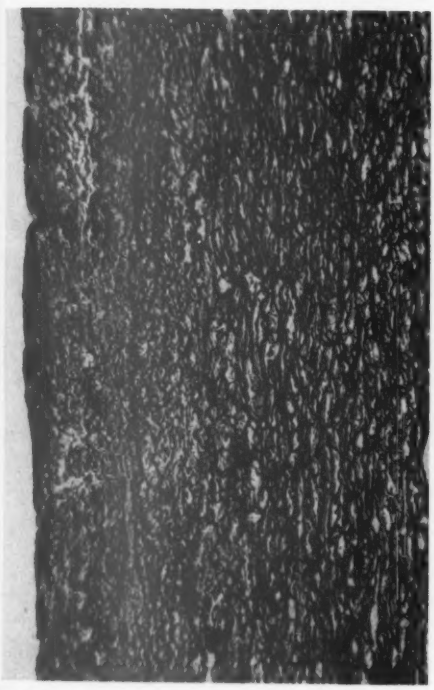

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8

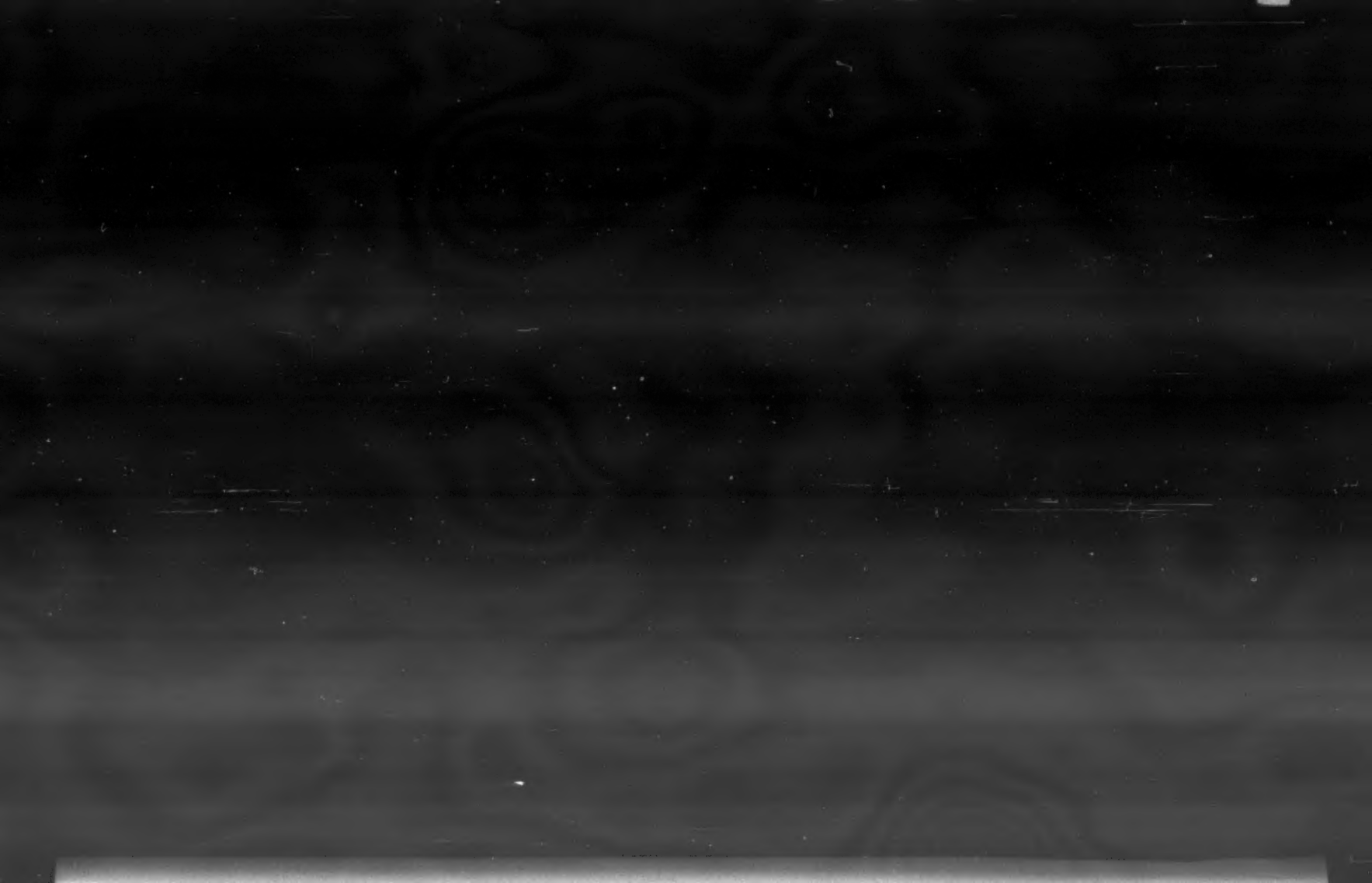
Wilens

Intimal Thickening of Arteries

PLATE 128

FIG. 9. Section of an aorta from a rabbit fed 3 gm. of cholesterol in olive-oil weekly for 16 weeks. The intima shows a uniform fibrous thickening comparable to that seen spontaneously in the human aorta. × 136.

FIG. 10. Low-power photomicrograph of the same rabbit's aorta shown in Figure 9. The fibrous intimal thickening is continuous and fairly uniform throughout the length of the vessel. × 8.



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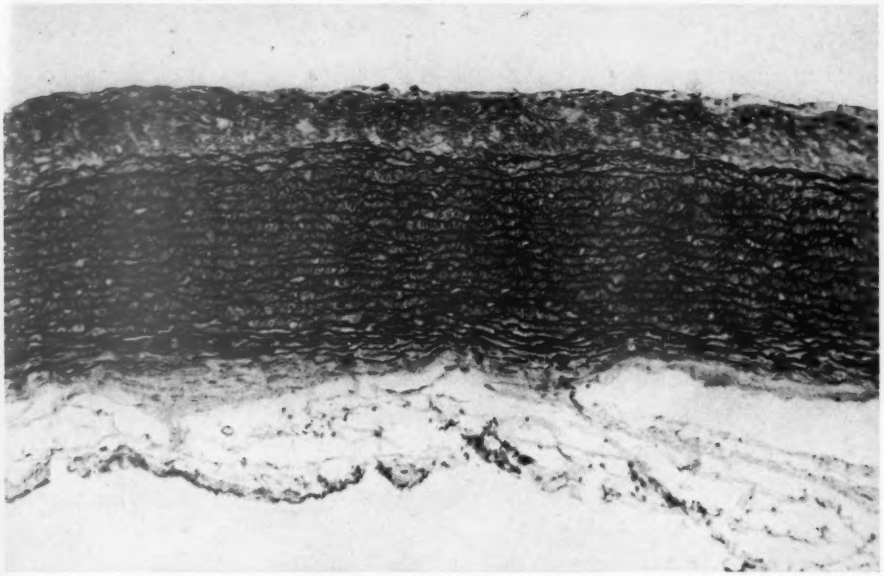

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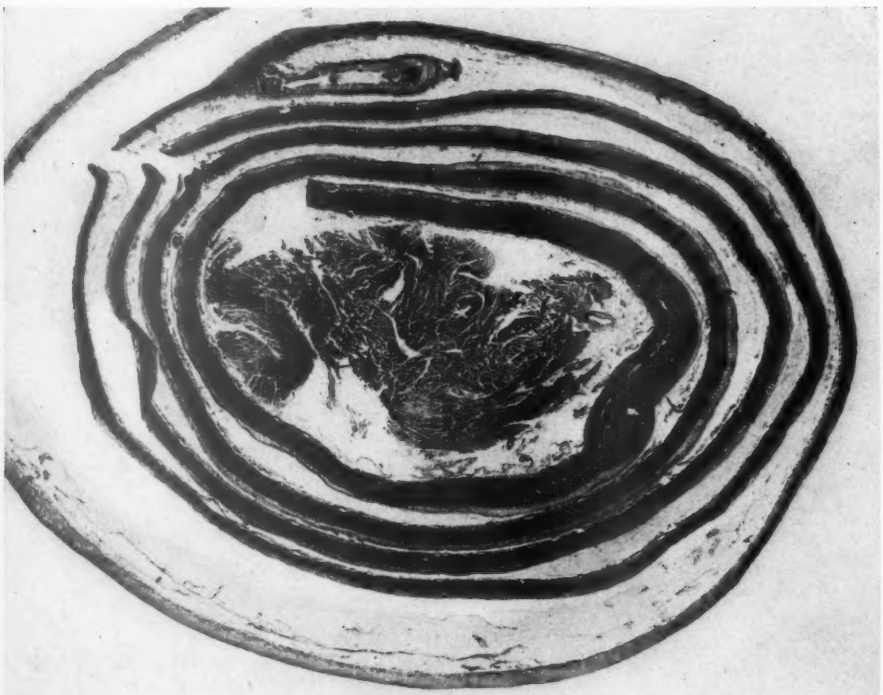

10

Wilens Intimal Thickening of Arteries

GLOMERULAR THROMBOSIS *

DAVID B. JONES, M.D., and WILLIAM E. LORING, M.D.
(From the Department of Pathology, State University of New York Medical Center at Syracuse, Syracuse, N.Y.)

Thrombosis of the capillaries of the glomerular tufts has been noted by many observers. It has been described in association with subacute bacterial endocarditis,[1-3] sepsis due to *Streptococcus pyogenes*[4] or to *Staphylococcus aureus*,[4] plague,[4,5] bacteremia with *Neisseria meningitidis*,[4] acute glomerulonephritis,[3,4] bilateral cortical necrosis of the kidneys,[6] lupus erythematosus disseminatus,[7] severe superficial burns,[8] thrombotic thrombocytopenic purpura,[9,10] and many other conditions. Since a concept of the pathogenesis of all cases of this condition has not been presented previously, an attempt is made in this paper to unify and clarify the concepts of this disease process.

Mallory[4] stated that glomerular thrombosis is part of acute intracapillary glomerulonephritis and that it represents the result of a more acute injury than that producing the usual types of acute diffuse proliferative glomerulonephritis. Forbus[11] placed these renal lesions in a group which he called acute focal glomerulonephritis, and differentiated this group as occurring during the height of a bacterial infection in contradistinction to the acute diffuse proliferative variety of glomerulonephritis, which occurs after the bacterial infective phase has subsided. Bell named these lesions focal (thrombotic) glomerulonephritis,[2,3] and stated that subacute bacterial endocarditis is the important cause of this lesion (52.8 per cent of his cases of subacute bacterial endocarditis showed glomerular thrombotic phenomena). Also he found that a few cases of acute bacterial endocarditis and rare cases of bacterial sepsis showed this lesion. In addition to focal (thrombotic) glomerulonephritis he mentioned the presence of glomerular capillary thrombi in the following cases: one of tuberculous peritonitis, one of congenital syphilis, 3 cases of rheumatic fever, 10 of 82 cases of acute proliferative glomerulonephritis, and 2 of 23 cases of subacute glomerulonephritis.

No previous attempt has been made to correlate the cases with hyaline glomerular thrombi having an obvious bacterial background with those in which a bacterial component is absent. All kidneys seen at necropsy in the Department of Pathology of the State University of New York Medical Center at Syracuse University from 1940 to 1949 were reviewed. Those showing hyaline glomerular thrombi were col-

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lected and analyzed. The cases were graded roughly into three groups: minimal, moderate, and massive glomerular thrombosis. In addition, 40 consecutive cases of bacterial endocarditis, starting in 1935, were analyzed to determine the incidence of glomerular thrombosis in these cases. All cases of acute diffuse glomerulonephritis occurring during the period 1940 to 1949 were studied for the same reason.

Methods

Case finding depended upon routine sections stained with hematoxylin and eosin. A characteristic clear red color of the hyaline thrombi aided in differentiating them from other red-stained material. Individual cases were studied by Foot's modification of Masson's trichrome stain. This stain was most satisfactory for the study of thrombi as they stood out as bright red masses against the yellow of the red blood cells. A slightly modified Weigert's fibrin stain, Mallory's phosphotungstic acid hematoxylin, and the periodic acid-Schiff's reaction were used frequently. Paraffin sections, 2 μ in thickness, were used in some cases for the study of the finer structure of the thrombi.

Incidence

In the period from 1940 to 1949, 82 cases of glomerular thrombosis were found in 2293 necropsies. This gives an incidence of 3.6 per cent of this condition in routine necropsy material. Only 7 cases in this group were associated with bacterial endocarditis. In Table I is shown the range of diseases with which this lesion was associated and also the severity of the renal involvement. Certain cases other than those of the period of study were used as supplemental material but are not included in Table I or in the calculation of incidence.

Analysis of Cases

In compiling the data on the collected cases it was obvious that they fell into six general groups: bacteremia, endocarditis, presumed bacteremia or toxemia, glomerulonephritis, systemic circulatory failure, and renal circulatory disturbance. The first four are directly or indirectly related to bacterial invasion and/or toxemia, and we believe that they should be termed the bacterial toxic group. As tissue anoxia secondary to systemic and/or local circulatory failure seemed to be the common feature in the last two groups, we believe that they might be called the anoxic group.

The Bacterial Toxic Group

Bacteremia. Nineteen cases comprised the bacteremic group and they consisted for the most part of overwhelming bacterial infections.

Incriminated organisms were *Diplococcus pneumoniae*, 6 cases; *N. intracellularis meningitidis*, 10 cases; *Staph. aureus*, 1 case; and organisms unidentified bacteriologically, 2 cases. The large number of infections with *N. meningitidis* was due to its almost epidemic occurrence during the war years. Ten of 16 cases of meningococcemia

TABLE I
Distribution of Cases

Diagnosis	Minimal	Moderate	Massive	Totals
N. meningitidis, sepsis	4	2	4	10
D. pneumoniae, sepsis	4	2	0	6
Staph. aureus, sepsis	1	0	0	1
Unknown organism, sepsis	1	0	1	2
Presumed bacterial infection	14	2	7	23
Hematogenous tuberculosis	2	0	1	3
Bacterial endocarditis	3	3	1	7
Pyelonephritis	1	0	0	1
Acute diffuse glomerulonephritis	0	3	1	4
Myocardial infarction	3	1	4	8
Circulatory failure	2	0	5	7
Renal infarction	2	1	0	3
Pulmonary infarction	2	0	0	2
Nephrosclerosis	0	1	2	3
Periarteritis nodosa	0	0	2	2
Totals	39	15	28	82

showed glomerular thrombosis. These cases have been reported previously.[12] Histologically, the renal lesions were essentially the same regardless of the pathogen involved (Figs. 1, 2, and 3). The glomerular tufts showed capillary thrombi and little or no endothelial proliferation. The thrombi had sharp, smooth edges and were not restricted to individual lobules of the glomerulus. The attachment of the thrombus to the endothelium was not seen clearly. The thrombi appeared to be made up of closely packed, longitudinally arranged fibrin fibrils with an occasional hollow center. Three cases with sepsis due to *Str. hemolyticus*, not included in the period of this study, showed essentially the same features except that pyknosis of endothelial cells near the thrombi was seen occasionally. Hematogenous tuberculosis was associated with glomerular thrombi in 3 instances.

Bacterial Endocarditis. Seven cases of bacterial endocarditis showed the glomerular thrombotic lesion. To gain a better understanding of this condition, 40 consecutive cases of bacterial endocarditis from previous years were studied and 14 cases showed glomerular thrombosis. In contrast to previous reports,[2,3] only 2 of 12 cases of subacute bacterial endocarditis showed the lesions of focal (thrombotic) glomerulonephritis. Other renal lesions seen in this additional series were acute glomerulitis (14 cases) varying from minor involvement up to full-blown, acute, diffuse, proliferative glomerulonephritis, 2 cases of subacute glomerulonephritis, and 10 of hematogenous pyelonephritis. The glomerular thrombi seen in the cases of bacterial endocarditis tended to be lobular in arrangement with the remainder of the glomerulus being relatively unaffected. This thrombosed lobule of the glomerular tuft usually was jammed with irregular, sometimes granular, hyaline thrombi. A few polymorphonuclear neutrophils and pyknotic nuclei often were seen in the immediate area, with actual necrosis of the lobule occurring in some instances. There was a notable tendency for the affected lobule to adhere to Bowman's capsule, and to have an epithelial crescent forming about it. In older glomerular lesions in the same kidney all traces of thrombi were gone; they had been replaced by loose or hyaline scar tissue (Fig. 4).

Presumed Bacteremia or Toxemia. Cases of presumed bacteremia or toxemia comprised the most heterogenous group. In none of these cases was definite sepsis known, yet each had, or may have had, a bacterial component which might be incriminated as the cause of the glomerular thrombi. Patients with infected cancers in a terminal stage, terminal bronchopneumonia, mesenteric thrombosis, leukemia, and similar conditions made up this group. Histologically, the lesions usually were like those in the bacteremic group but occasional cases showed the peculiar lobular character of the bacterial endocarditic group. In several cases a striking thrombosis of the preglomerular arterioles was present.

Diffuse Glomerulonephritis. Since the relationship of bacterial infection to diffuse glomerulonephritis is obscure, the group of cases with diffuse glomerulonephritis is considered separately. Of 12 cases of acute diffuse glomerulonephritis occurring during the period of study, only 4 were found to show hyaline glomerular thrombi. The cases of acute, proliferative, intracapillary glomerulonephritis were singularly free of thrombi except for one which had extremely acute, exudative arteritis of all small renal arteries. On the other hand, all 3 cases of acute, capsular, proliferative glomerulonephritis showed irregular hyaline thrombi in glomerular capillaries and fibrin clots amid the pro-

liferating capsular epithelium. The marked evidence of hemorrhage and exudation into the proximal convoluted tubules, and the capillary thrombi suggested a far more severe injury to the glomerulus in the latter variety of nephritis.

One case of chronic pyelonephritis showed thrombi which resembled those of the bacteremic group.

The Anoxic Group

Systemic Circulatory Disturbance. In 24 cases in which glomerular thrombi were present, there was little or no evidence of bacterial infection. For the purposes of clarification this group of cases has been divided into two subgroups: systemic circulatory failure and renal circulatory disturbance. This division is confused in view of the work of Trueta and his associates [18] who demonstrated the severe local ischemia of the kidney that is produced by a general disturbance of the circulatory system. Myocardial infarction, severe congestive heart failure, shock, dehydration, and pulmonary infarction were found to be associated occasionally with glomerular thrombosis. Myocardial infarction was the most prominent single cause for thrombi in the anoxic group, contributing 8 cases. It is believed that endothelial anoxia is the necessary feature for the occurrence of thrombosis and that renal ischemia mediated in a manner such as described by Trueta is the mechanism of ischemia. Histologically, the thrombi do not differ significantly from the smooth thrombi seen in bacteremia (Figs. 5 and 6). Focal necrosis of tubules in the region of an involved glomerulus was seen occasionally.

Renal Circulatory Disturbance. The renal circulatory group consisted of cases in which there was local kidney disease sufficient to explain glomerular thrombosis, although systemic circulatory disturbances may well have played a significant part in the pathogenesis. Three cases of renal infarction were placed in this group. Grossly, there were no sources of emboli; thrombosis of larger renal vessels was not demonstrated at the time of necropsy; and there was no evident systemic cause for the infarction. Certain cases included under other categories showed infarction, but those cases had systemic diseases which, it was believed, produced renal ischemia that resulted in endothelial injury and thrombosis of arterioles and glomerular capillaries. Glomerular thrombi were seen primarily in ischemic necrosis, *i.e.*, in kidneys in which the blood flow was sufficient to support the stroma but the tubules were infarcted. In true complete infarction, capillary thrombi were seen only rarely and then only at the very periphery of the infarct.

Two cases of benign arteriolar nephrosclerosis and 2 cases of malignant nephrosclerosis, one of which is not included in the period of study, showed widespread glomerular thrombi. Histologically, the thrombi tended to be somewhat granular and irregular. The preglomerular arterioles frequently were the site of thrombosis and necrosis and, in these cases, this is believed to be of prime importance in the pathogenesis of the glomerular thrombosis. Two cases of periarteritis nodosa showed glomerular lesions closely resembling those of malignant nephrosclerosis.

Discussion

Source of the Thrombi

It has been shown that endothelial injury is the prime prerequisite for intravascular thrombosis.[14] With slight injury there is stasis, and platelets, red blood cells, and white blood cells adhere to the injured areas; but with cessation of the injurious influence the cells loosen and are washed away. With a more severe injury the clotting mechanism is initiated and fibrin is formed. The formation of fibrin threads in the minute lumen of the capillary in the presence of blood flow would tend to form a thrombus tail which would consist of longitudinally arranged strands of fibrin cemented together by platelets. This theory of formation of the capillary thrombi is the one to which we subscribe. In the past these thrombi have been thought to be due to the intravascular agglutination of red blood cells.[15] The negative results with hemoglobin stains, the positive results for glycoproteins by the Hotchkiss stain, which is negative for red blood cells but positive for fibrin and platelets, and the structure of the thrombi as seen by Weigert's fibrin stain or Mallory's phosphotungstic acid hematoxylin, which is that of sheaves of fibrin threads, are strong proof that the red cells are not implicated (Fig. 3). Some of the thrombi show a granular structure which indicates that platelets contribute a large part to the formation of at least some of them. In paraffin sections cut at 6 to 8 μ and stained with Weigert's fibrin stain or with Mallory's phosphotungstic acid hematoxylin without prolonged staining, the thrombi may be colored a pale blue by the former and a pale brown by the latter. This is of interest as Fitzgerald and his associates [10] used a staining reaction such as this as proof that the thrombi of thrombotic thrombocytopenic purpura are made up of platelets. With prolonged staining of these thicker sections the characteristic staining of the fibrin was obtained. In control studies of ante-mortem thrombi of large vessels, it was believed that agglutinated platelets stained in the same manner as fibrin, using the special stains mentioned previously. The use of paraffin

sections 2 μ in thickness permitted a sharpness of staining and clearness of structure which were found to be necessary in the evaluation of the thrombi.

Functional Significance

A series of 27 cases showing moderate to severe glomerular thrombosis and having clinical data available was studied in order to determine some of the clinical implications of this renal thrombotic process (Table II). These patients tended to show somewhat bizarre clinical

TABLE II

Clinical and Laboratory Findings in 27 Cases of Moderate to Severe Glomerular Thrombosis

Diagnosis	Blood pressure	Non-protein nitrogen	Urinary specific gravity	Urinary albuminuria	Urinary red blood cells per high-power field	Hemorrhagic lesions
	mm. Hg	*mg. per 100 cc.*		*grade*		
Sepsis	120/90		1016	2	Occasional	o
Dissecting aneurysm	260/150	125	1022	4	Many	+
Myocardial infarction	200/120	75	1018	1	o	o
Bacterial endocarditis	138/66	150	1014	4	20	o
Myocardial infarction	146/110	25		1		o
Nephrosclerosis	260/150	73	1015	4	Occasional	+
Enteritis	140/80	40	1017	4	Occasional	o
Wound infection	160/100	35	1011	1	o	o
Otitis media	115/65	110	1013	4	Many	o
Pulmonary infarction	104/60		1013	2		o
Sepsis, *N. meningitidis*						+++
Nephrosclerosis	220/125	115	1015	2	Many	+
Carcinoma of lung	140/100	40	1020	2	Few	o
Lung abscess	105/80	29	1013	3	300	+++
Carcinoma of cervix	132/82	72	1020	2		o
Periarteritis nodosa	130/80	77	1016	3	20	o
Chronic glomerulonephritis	136/80	43	1024	4	20	o
Bronchopneumonia	100/60		1016	2	Many	+
Myocardial infarction	250/115	106		3	Many	o
Bronchopneumonia	228/126	90	1010	1	Rare	o
Myocardial infarction	110/95		1012	Trace	o	o
Bilateral renal cortical necrosis	170/90	104	1017	o	o	o
Malignant nephrosclerosis	230/170	94	1021	4	Rare	+
Acute glomerulonephritis	160/100		1015	o	o	o
Acute glomerulonephritis	120/80	223		4	o	o
Shock and dehydration	104/60			1	o	o
Sepsis, *Strep. pyogenes*						++

pictures with renal failure and occasional purpuric hemorrhages as the prominent features. Blood pressure determinations varied widely, as might be expected from the heterogenous diseases associated with the renal lesion, and did not appear to be significant. Many of the patients were febrile but no specific significance could be attached to this fact other than that many suffered from bacterial infection. Hemorrhagic lesions were seen in 8 cases, and in 3 cases, including one case of meningococcemia, prominent purpuric skin lesions were seen. In sub-

acute bacterial endocarditis, Bell [3] has said that uremia may occur from renal involvement. In most cases it is due to diffuse proliferative glomerulonephritis, but occasionally the only prominent renal lesion to incriminate for the uremia is glomerular thrombosis. An elevation of the non-protein nitrogen of the blood was found in 13 of 19 cases in which it was determined. Albuminuria was found in 23 of 25 cases in which suitable tests were performed, and in 11 cases was 3 or 4 plus. Of 22 cases in which such an observation was made, 15 showed red blood cells in the urine and in 9 cases they were numerous.

Fate of the Thrombi

It is conceivable that thrombi consisting largely of platelets may disappear by the reversal of the equilibrium of the physiochemical process of agglutination, but with fibrin formation this is unlikely. The fate of the fibrin thrombi is obscure, but several possibilities may be suggested: intravascular phagocytosis and lysis by mononuclear cells, fibrinolysis by enzymes present in the blood, or, most likely, organization of the material in a manner like that seen in a thrombus of a large vessel. It is difficult to conceive of severe glomerular thrombosis resulting in other than scarred or partially scarred glomeruli. Bell [3] stated that the thrombi in focal (thrombotic) glomerulonephritis do not result in fibrous glomeruli, but that the involved tufts disappear by lysis. He stated that the fibrous glomeruli in these kidneys have a different pathogenesis, comparable to that of subacute glomerulonephritis. We believe that the hyaline masses, which Bell interpreted as disintegration of glomerular lobules, with complete loss of glomerular structure, are in most cases masses of clotted fibrin lying in Bowman's space, and that the fibrosed glomeruli seen in the same kidney in subacute bacterial endocarditis are the end stage of the process of healing of these thrombotic lesions (Fig. 4). In most cases other than of bacterial endocarditis in this study, the injury apparently occurred at one time rather than in repeated episodes, as in bacterial endocarditis, and thus the glomerular lesions are all in about the same stage of development. This makes it difficult to determine what the end result of these lesions would have been if the patient had survived. Ten of 16 cases of sepsis due to *N. meningitidis* showed glomerular thrombi, yet in surviving cases of meningococcemia renal impairment has not been seen. This may suggest that the lesion is reversible; that, through treatment which saved the patient, widespread glomerular thrombi did not form; or that those with the most severe renal thrombosis died and the survivors did not have a sufficient loss of glomeruli to impair renal function clinically. It is believed that if it were possible to examine the kidneys of

such patients who survive, they would reveal signs of glomerular scarring, but of insufficient amount to interfere seriously with kidney function, since in the majority of our cases less than 50 per cent of the glomeruli were involved with thrombi. In addition it is believed that the scarred glomeruli in such kidneys would resemble those of chronic glomerulonephritis or pyelonephritis. Occasionally in neonatal kidneys one sees partially or completely hyalinized glomeruli with slender epithelial crescents along with fresh glomerular thrombosis. This is additional confirmatory evidence that the old lesion of glomerular capillary thrombosis is a hyaline scar resembling glomerulonephritis.

Systemic Involvement

A series of 30 cases with severely involved kidneys was studied in order to determine the extent of thrombosis in other viscera. Table III indicates the distribution of other capillary thrombi. Twenty-four of the 30 cases showed some extrarenal capillary thrombi, but the predominant organs involved were the lungs (Fig. 7), liver, and spleen (Fig. 8). With the large capillary bed in these organs, it can be questioned whether any functional significance should be assigned to the presence of the thrombi. A terminal bronchopneumonia in some of the cases beclouded the significance of the pulmonary thrombi since they can occur in severe pneumonitis. When skin or mucous membranes were involved with capillary thrombi, focal hemorrhages usually were present.

In 14 of 16 cases of sepsis due to *N. meningitidis,* some of which are included in this series, Ferguson and Chapman [12] found capillary thrombi in multiple viscera. Only one of these cases is included in Table III in order not to give an unfairly large percentage of visceral involvement.

Visceral capillary thrombosis is of particular interest in relation to the cases of disseminated platelet thrombosis associated with thrombocytopenic purpura, fever, and diffuse clinical signs. Pathologically, these cases are characterized by widespread hyaline thrombosis of capillaries and arterioles of many viscera. It has been postulated that thrombocytopenia in these cases is due to the massive removal of platelets from the circulation by this thrombotic process. Purpuric lesions were seen in 8 cases in our series. In most of them a bacterial basis for the purpura was likely, but in most of the cases it was not proved. Unfortunately, platelets were not studied in our cases during life. In one case in which the course was not so fulminant, a considerable similarity to thrombotic thrombocytopenic purpura was seen. The capillary thrombotic process which this paper discusses is rela-

TABLE III

Distribution of Visceral Capillary Thrombi

Diagnosis	Kidneys	Lungs	Liver	Heart	Spleen	Brain	Urogenital tract	Gastro-intestinal tract	Others
Chronic glomerulonephritis	++								
Acute glomerulonephritis	++	+			+				
Acute glomerulonephritis	++								
Acute glomerulonephritis	+++							+	+
Lung abscess	+++	++			++			+	
Bronchopneumonia	+++	++			+			+	+
Myocardial infarction	+++								
Myocardial infarction	+++	+							+
Myocardial infarction	+++								
Shock and dehydration	+++	+		+	+	+			+
Sepsis	+++	+							+
Dissecting aneurysm	+++	++	+	+					
Carcinoma of cervix	+++	++			+		+	++	
Bronchopneumonia	++							+	
Otitis media	+++	+	+		+		+		+
Myocardial infarction	+++		+						
Bilateral cortical necrosis	+++	+							
"Cold"	+++	+		+		+			
Nephrosclerosis	++	+	+						
Periarteritis nodosa	+++	+							
Pulmonary infarction	+						+	+	
Enteritis	+++	+				+		+	
Wound infection	+++	+							+
Nephrosclerosis	+++								
Streptococcal sepsis	+++				+				+
Meningococcemia	+++								+
Carcinoma of bronchus	+++	+	+						+
Acute bacterial endocarditis, *Staph. aureus*	+++								
Myocardial infarction	+++	+	+					+	
Malignant nephrosclerosis	+++	+							

tively common, while the disease thrombotic thrombocytopenic purpura is very rare, as one would gather from the number of reported cases. We feel that the same basic pathologic process is involved in both conditions and that there are transitions between the two processes. Thus thrombotic thrombocytopenic purpura becomes merely an unusually severe variant of the relatively common process of capillary thrombosis.

Summary

Study of the kidneys of 2293 consecutive necropsies revealed 82 cases of hyaline thrombosis of the glomerular capillaries. These cases were divided into bacterial toxic, and anoxic groups. Endothelial injury, whether due to bacteria or anoxia, is thought to be the cause of thrombosis. It is postulated that obliteration of capillaries with scarring is the end result of healing of these lesions. Capillary thrombi in viscera other than the kidney were found in 24 of 30 cases in which the entire necropsy material was studied especially. It is suggested that the disease thrombotic thrombocytopenic purpura is an unusually severe variant of this pathologic process.

Grateful acknowledgment is due Miss Stella Zimmer and Louis Georgianna for the illustrative material.

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DESCRIPTION OF PLATES

PLATE 129

FIG. 1. Massive glomerular capillary thrombosis in a case of sepsis due to *Neisseria meningitidis*. Goldner's modification of Masson's trichrome stain. × 325.

FIG. 2. Glomerular capillary thrombosis in sepsis due to *Streptococcus pyogenes*. Periodic acid-Schiff's reaction. × 310.

FIG. 3. Post-partum sepsis secondary to acute otitis media with massive glomerular, hyaline, capillary thrombi. The sheaves of fibrin threads are apparent. Mallory's phosphotungstic acid hematoxylin stain. × 415.

FIG. 4. Bacterial endocarditis, organism unknown, with fresh and organized thrombi of glomerular lobules. Of note are the granular, fresh thrombi in the middle lobule, and the fibrous, organized lobule beneath it. Foot's modification of Masson's trichrome stain. × 415.

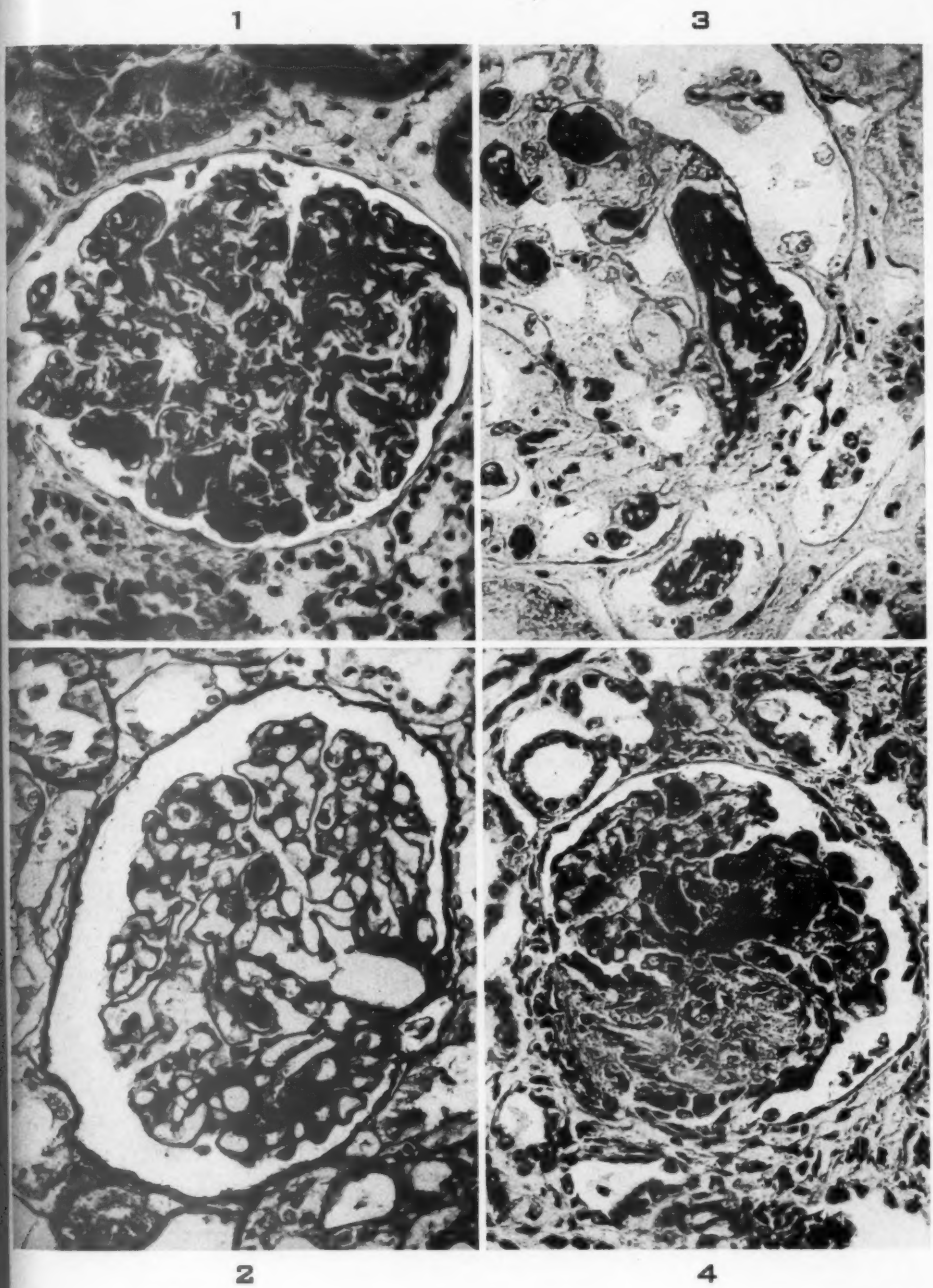

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PLATE 130

FIG. 5. Glomerular thrombosis associated with heart block and congestive heart failure. Periodic-acid Schiff's reaction. × 310.

FIG. 6. Thrombi in vasa recti of the kidney associated with dehydration, shock, and massive glomerular thrombosis. Weigert's fibrin stain. × 620.

FIG. 7. Thrombosis of pulmonary capillaries in a case of old dissecting aneurysm and congestive heart failure showing glomerular thrombosis. Foot's modification of Masson's trichrome stain. × 620.

FIG. 8. From the same case as Figure 3, showing thrombosis of splenic sinusoids. Foot's modification of Masson's trichrome stain. × 385.

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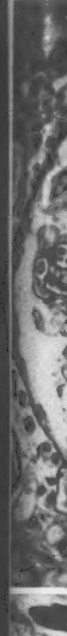

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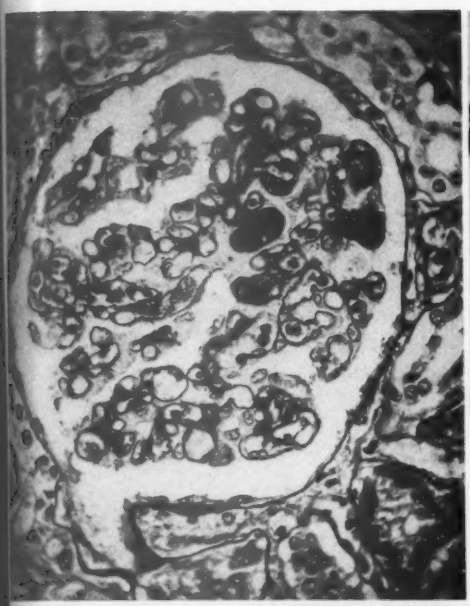

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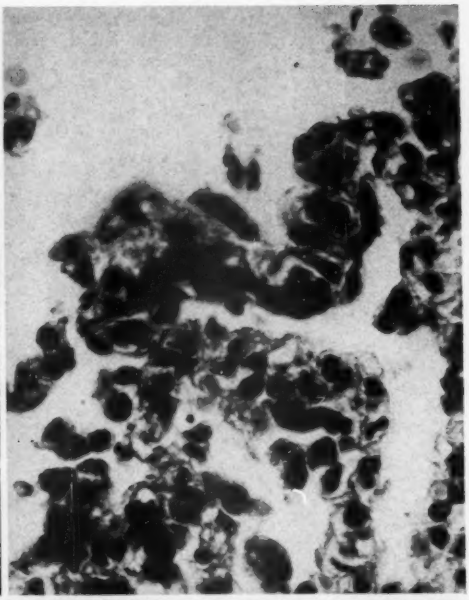

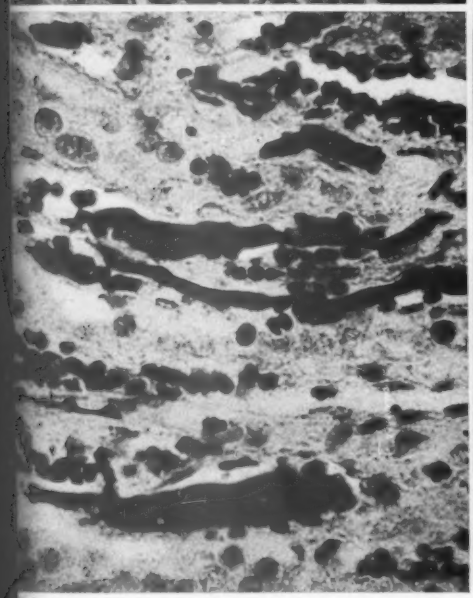

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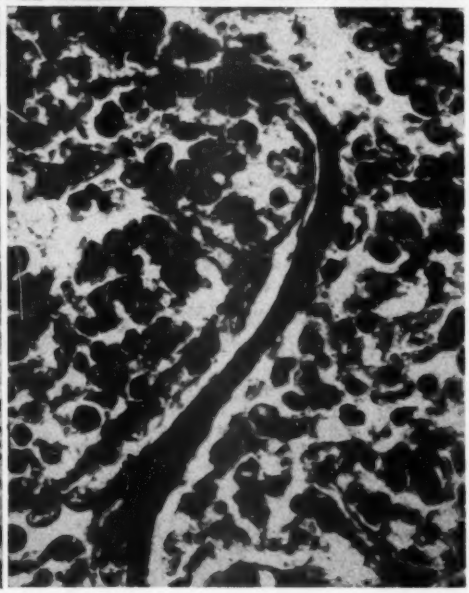

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THE RENAL LESIONS ASSOCIATED WITH EXPERIMENTAL DIABETES IN THE RAT *

GEORGE V. MANN, M.D., JAMES W. GODDARD, M.D., and LUCILE ADAMS, A.B.
(From the Department of Nutrition, Harvard University School of Public Health, Boston 15, Mass.)

The work reported here is a part of an investigation of the problem of arteriosclerosis. The use of diabetic experimental animals in the study of this problem is predicated upon the well known clinical observation that diabetic humans develop arteriosclerosis at an accelerated rate.[1,2] Since lack of a suitable experimental animal for the study of arteriosclerosis has handicapped progress in this field, we have investigated the influence of diabetes upon the vessels in several species of laboratory animals. In this report, work with alloxan-induced diabetes in the albino rat will be described.

Lukens and Dohan [3] have described morphologic changes in the kidneys of a single dog kept diabetic with anterior pituitary substance for 5 years. These changes appear to be a reproduction of the renal lesions of intercapillary glomerulosclerosis which is frequently seen in humans with diabetes of long duration.[4-6] While our work was in progress, Foglia, Mancini, and Cardeza [7] described diffuse and nodular changes in the kidneys of partially pancreatectomized diabetic rats which they believed to be the equivalent of human intercapillary glomerulosclerosis.

PROCEDURE

Weanling male rats of the Sherman strain were made diabetic by the intravenous administration of alloxan.[8] Young animals were used in an attempt to simulate "juvenile diabetes" in humans and to avoid the cumulative effects of other "natural" diseases which occur with age; in the rat this is notably pyelonephritis.

Each experimental group included alloxan-injected animals with a degree of diabetes ranging from severe through mild to no demonstrable diabetes, as well as non-injected controls. This procedure allows at least partial control of tissue damage due to alloxan which is not mediated through diabetes. Groups of usually 10 animals were kept in screen-bottomed cages in rooms maintained at 24° C. At intervals of 1 to 4 weeks alloxan-treated animals were placed for 48 hours in individual metabolism cages and quantitative collections of

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urine were made with thymol as a preservative. Urine glucose was measured by a photometric adaptation of the Somogyi procedure.[9] The animals were maintained on one of several diets, the compositions of which are described in Table I.

Animals were sacrificed at intervals for histologic study. Those found dead were discarded as useless. Tissues were fixed in both

TABLE I

The Percentage Composition of Experimental Diets

Diet	P-10	P-20	P-40	F-30	Chow (Gaines dog meal) (mfr. analysis)
	%	%	%	%	%
Casein (crude)	10	20	40	20	Protein, 25.1
Corn oil (mazola)	5	5	5	30	Non-fiber extract, 49.6
Sucrose	68	58	38	33	Fat, 6.6
Brewers' yeast (Anheuser-Busch, strain K)	10	10	10	10	Fiber, 2.9
Salts IV[10]	4	4	4	4	Ash, 8.6
Cod-liver oil U.S.P.	3	3	3	3	

Zenker's fluid and Lillie's buffered, neutral formalin. After paraffin embedding the kidney sections were stained routinely with Masson's trichrome, Mallory's phloxine and methylene blue, and with the McManus periodic acid-leukofuchsin technic.

This study involved 438 animals over a period of 3 years. Animals have been maintained up to 30 months before necropsy. Since no insulin was given, the animals of long survival are, in general, not those with the most severe diabetes. All experiments have been repeated at least once.

RESULTS

Depending upon the severity of the diabetes and the nature of the diet, growth in the diabetic animals was retarded. Occasional non-glycosuric animals showed retarded growth. In those instances, glucose tolerance tests revealed curves of the diabetic type. Control animals became obese as did alloxan injected, non-diabetic animals. It was apparent that the diabetic animals were severely restricted in available calories and were small in consequence. This may be of importance because of the suggestive correlations between obesity and diabetes and between diabetes and vascular sclerosis in humans.

The diabetic animals regularly developed cataracts. This and other metabolic aspects of the relationship between diet and diabetes will

be described in a later paper. It is perhaps worth comment that the diabetic animals were especially susceptible to infection. Bacterial pulmonic diseases and pyelonephritis were common causes of death. In addition, skin infections of the feet and infections of the prepuce were common in the debilitated animals. At necropsy, abscesses were found frequently in the lungs, liver, or around the genital organs. In severely diabetic animals (with fasting blood sugar levels of 300 mg. per cent or more), glycogen deposition in the distal convoluted tubules of the kidney was found routinely.

Gross and microscopic examination of the aortas yielded no evidence of atheromatosis or other injury. The muscular arteries appeared normal even after 24 to 30 months of diabetes, and irrespective of the diet or severity of the diabetes. The gross appearance of the kidneys has been within the normal anatomical range. Histologic examination revealed one significant vascular lesion. This was a sclerosing process involving reticulin structures in the renal glomerulus, and this will be described in detail.

The principal vascular lesion consisted of changes in the reticular pattern of the glomerular capillaries. This lesion appeared as reticulin proliferation centered in the glomerular tuft which progressed to a sclerosing, obliterative disorder, eventually destroying the normal glomerular architecture completely. For description, this lesion may be divided into two components: fibrillar and cellular.

Fibrillar Changes

The fibrillar changes always preceded the cellular. The walls of the glomerular capillaries were affected primarily. The process began as a localized thinning of the capillary wall resembling a local area of erosion, which progressed in some instances to an actual defect with extravasation of blood into the intercapillary space (Fig. 1). These sites then became points of repair with migration of histiocytes and reticulin proliferation. More commonly, the initial observed change was an aberration in the staining of the reticulin component of the capillary walls, the glomerular supporting matrix, or of the parietal layer of Bowman's capsule. Initially, these aberrations also were localized. The areas became more eosinophilic and stained more intensely with the periodic acid-leukofuchsin stain. This initial change was then gradually supplanted by the most characteristic morphologic alteration in the kidneys of diabetic rats—the appearance of hyaline masses infringing upon and obliterating the normal glomerular structure.

There followed a remarkable increase of reticulin fibers which not only became more numerous but also thicker, more profusely branching and arboreal in pattern (Fig. 2). Variable involvement of the capillary walls led to an irregular contour with thickening, or, in some places, reduplication. The reticulin overgrowth might become intraluminal causing obstruction of the loop, or the pericapillary involvement so intense that capillaries were obstructed by a sphincter of encircling reticulin fibers (Figs. 6 and 11), the contour assuming an hourglass configuration. Characteristically, the reticulin overgrowth led in either case to obstruction and the remainder of the capillary loop was empty as to blood cells, although held rigidly distended by the surrounding matrix (Figs. 3 and 13). In their pericapillary outgrowth, the newly formed reticulin fibrils coursed over the mesangium, imparting to the latter a fenestrated appearance (Fig. 11). Even when not pervaded by reticulin fibrils, the mesangium in the experimental animals appeared more conspicuous than in the controls, by its increased density and proliferation (Fig. 14).

The pericapillary reticulin fibers extended to neighboring capillaries and led to fusion with a distinct alteration of the normal glomerular pattern of lobulation (Fig. 4). This fusion might involve a few capillary loops or all of the glomerular "lobes." If only a few loops were involved, the result was a fibrillar mass or "ball" (Fig. 6). If all were involved, the entire glomerulus was converted to a functionless, fibrous mass, which ultimately might become hyalinized (Figs. 2 and 8). In the rat these masses did not assume the collagen stains as do the lesions of intercapillary glomerulosclerosis in the human being. This represents a notable difference from the human lesion.

The same process was responsible for the fusion of capillary loops with the Bowman capsule (Figs. 11 and 12). In some instances fibrils growing out from the capillary wall coalesced with reticular fibrils of Bowman's capsule (Fig. 10). In other instances fibrils splitting from Bowman's capsule grew toward either the nearest loop or to a fibrillar network which surrounded such loops (Fig. 3).

The primary participation of the reticulin fibers from Bowman's capsule intimates that the effect of diabetes is not solely upon the capillaries *per se* but upon the reticulin.

Cellular Changes

The endothelial cells in the affected glomeruli were increased in number. These proliferating cells might appear stellate and mobile or

by juxtaposition appear epithelial. In the functionless capillaries they became freed from their attachments and grew freely in a spreading pattern (Fig. 12). Finally, as the glomerulus filled with reticulin matrix and fibrils, their nuclei became pyknotic and the endothelial derivatives eventually disintegrated (Figs. 13 and 15).

The perithelial cells appeared both hyperplastic and hypertrophic in the affected kidneys. Although the origin of the reticulin fibers was obscure, we considered the reticulin proliferation to be under the control of these hyperplastic perithelial cells because of the parallelism of events. The failure of collagenization of the reticulin fibers so consistently seen in the diabetic rat might be related to the failure of these perithelial cells to evolve toward fibroblasts. The perithelial cells survived the endothelial cells in the involved glomeruli and finally assumed a granular appearance and were last seen as cellular débris within and around the hyalinized masses of the glomerular remnants (Fig. 15).

The cells lining Bowman's capsule showed hyperplasia (Figs. 11 and 15). Some appeared to exfoliate and to penetrate between the glomerular capillary loops, contributing to the formation of adhesions. The capsular membrane became thicker and heavier (Figs. 1, 3, 5, 8, 10, and 11) and there, in distinction from the glomerular tuft, collagenization frequently was seen in the late stages of the disease.

Although most of the intercapillary masses could be retraced to an overgrowth of reticulin matrix and reticulin fibrils, for some of the hyaline balls, which appeared in the capillary loops, a different origin was indicated. Fuchsinophilic globules might appear either intraluminally or in pericapillary positions (Figs. 4, 11, and 14). They were seen frequently in the angle formed by two or several neighboring loops, engulfed in the mesangium or harbored by the epithelial cells (Figs. 11 and 14). These spherules might conglomerate into irregular clumps, which filled in the entire space between the capillaries. They showed varying degrees of staining density (with the periodic acid-leukofuchsin stain) and some tinctorial selectivity with a Masson stain (most of the clumps taking the fuchsin or the ponceau components, but some being stained in shades of blue or orange). They usually were embedded in a fuchsinophilic sheet which stained lighter than normal and which appeared more homogeneous or glassy. Spherules, clumps, and the matrix in which they occurred formed intercapillary masses which might or might not be pervaded by fibrils (Fig. 14). They finally underwent hyalinization.

We have searched particularly for arteriolar lesions in these experi-

mental animals. Blood pressure measurements in selected animals by the indirect method of Grollman have not revealed hypertension. Neither has there been evidence of cardiac hypertrophy. In some animals we have seen minimal evidence of muscular hypertrophy in arterioles, particularly in the myocardium, adrenal capsule, and testes. These arteriolar changes have not been seen in the stage of medial hyalinization or of necrotizing arteritis. Whether this minimal hypertrophy of the muscular element of the arterioles is analogous to that described by Moritz and Oldt [11] in human "essential hypertension" is uncertain. Certainly the almost universal observation of arteriolar disease in conjunction with glomerular changes in human kidneys with intercapillary glomerulosclerosis is not true of the rat under these experimental conditions.

In a previous publication,[6] the correlations between the presence of intercapillary glomerulosclerosis in human diabetic patients and the severity and duration of diabetes have been described. In the present experiments a study was made of similar possible correlations in the diabetic rat and also of the relationship of composition of diet to the development of renal lesions. The lesions observed were graded according to degree of involvement into four groups. Representative sections were examined and the involved glomeruli enumerated and graded. Usually 75 to 100 glomeruli were counted for each animal. Scatter diagrams relating the incidence of these lesions to the duration of diabetes, severity of diabetes, and the nature of the diet failed in all instances to reveal significant correlations.

From this analysis it appears that the earliest fibrillary changes begin 25 to 50 days after the induction of diabetes. The disease process then develops slowly and in the course of 3 to 6 months comes to involve almost all glomeruli to some degree. However, the rate of development in different animals as well as the rate of development in different glomeruli appears variable and cannot be correlated with our experimental measurements. The frequent coincidence of pyelonephritis with this glomerular lesion suggests a correlation, but it has not been possible to measure the relationship.

Discussion

The histologic characteristics of the renal lesion in diabetic rats show many resemblances to human intercapillary glomerulosclerosis. The absence of hypertension and the failure of the lesion to develop in non-diabetic control rats suggest strongly that the disease is caused by diabetes. The experience of Foglia *et al.*[7,12] with rats made diabetic

by partial pancreatectomy further supports the contention that this lesion is caused by diabetes.

The lack of collagenization and the absence of arteriolar disease in conjunction with this renal lesion are two prominent differences between the rat and man. We have been unable to define conditioning factors, other than the presence of diabetes, which influence the disease. There is suggestive evidence that the bacterial infection which is common in diabetic rats may accelerate the process. Similar evidence has been proposed in relation to the human renal lesion of diabetes.[2]

The recent demonstration by Duff and McMillan [13] and by ourselves [14] that diabetes tends to lower plasma cholesterol levels and to prevent atherosclerosis in experimental animals may explain the absence of arterial lesions in these animals. While there is disagreement among authorities, it would appear that insulin therapy has had no influence upon the prevalence or severity of vascular disease in human diabetics.[6] Before arteriosclerosis can be eliminated as a complication of diabetes in the rat, experiments must be conducted in which diabetic rats are maintained on adequate daily doses of insulin in order to prevent caloric insufficiency.

The characteristic renal lesions of diabetic rats, described here, provide a useful experimental tool for the study of the pathogenesis of human intercapillary glomerulosclerosis, which we believe to be analogous.

Summary

Weanling male rats were made diabetic with alloxan and studied for periods of 1 to 30 months for the development of cardiovascular lesions. Controlled groups of animals were kept on one of five types of diet varying in the content of protein and fat.

No significant lesions were found in the heart or large vessels. A distinctive lesion was found in the renal glomerular capillaries. This lesion consisted of a progressive alteration in reticulin structures. The changes were a proliferation of reticulin fibers leading to an obliterative, sclerosing process which progressed eventually to destruction of the entire glomerulus.

Unlike lesions of the human disease intercapillary glomerulosclerosis, there was minimal collagenization in the injured glomerulus. Neither was significant arteriolar disease apparent. In these experiments it was not possible to relate the severity or duration of diabetes to the renal lesions. The nature of the diet could not be shown to have influenced the lesions.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 131

FIG. 1. N 290–50. Duration, 7 months; diet, F–30; severity, 3.2 gm. of urinary glucose per 24 hours. On the left are eroded capillaries with extravasated red blood cells between the loops. Dark patches of fibrils and reticulin matrix are apparent. A ball in formation at the expense of capillaries near Bowman's membrane is seen at the top of the right-hand corpuscle. Zenker's fixation, periodic acid-leukofuchsin and Weigert's iron hematoxylin stains.

FIG. 2. N 290–50. Same specimen as Figure 1. Almost all capillaries are obliterated. The remaining patent capillaries have narrowed lumina with thickening of reticulin in their walls. The endothelial and epithelial cells appear viable, although the glomerulus is functionless and is being transformed into a mass of reticulin fibrils.

FIG. 3. N 48–49. Duration, 4 months; diet, P–40; severity, 2.0 gm. of glucose per 24 hours. The capillary lumina are visible but bloodless and are filling with reticulin material. There is collagenization of the central lobule and marked thickening of Bowman's membrane. Zenker's fixation, periodic acid-leukofuchsin and Masson's trichrome stains.

FIG. 4. N 19–50. Duration, 6 months; diet, P–40; severity, 1.2 gm. of glucose per 24 hours. Intraluminal and pericapillary fuchsinophilic granules with two hyaline masses. Zenker's fixation, periodic acid-leukofuchsin and Masson's trichrome stains.

FIG. 5. N 19–50. Same specimen as Figure 4. This corpuscle illustrates the collapse of capillaries with an increase of parietal and capsular reticulin.

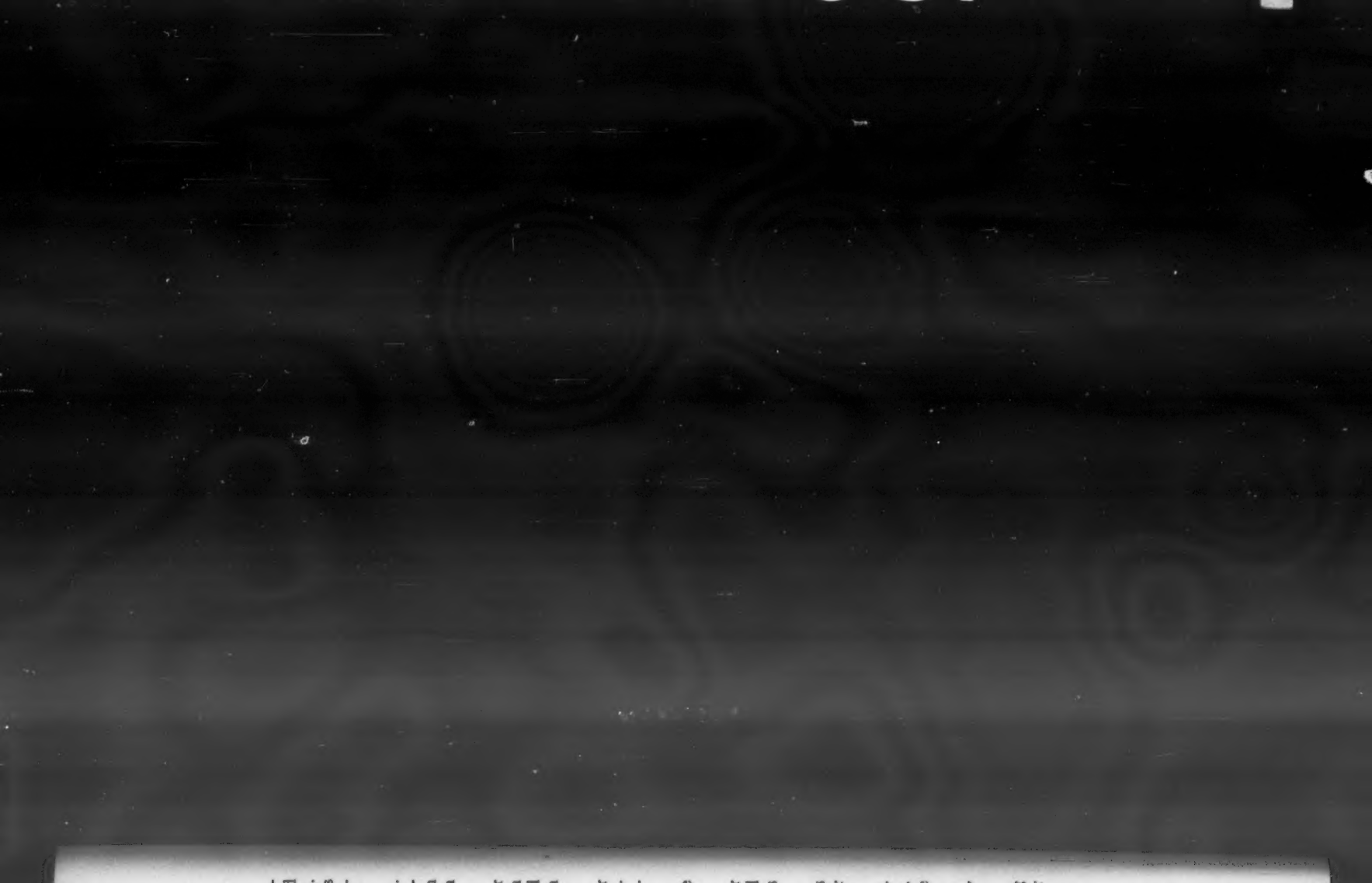
FIG. 6. N 17–50. Duration, 3 months; chow diet; severity, 3.8 gm. of glucose per 24 hours. An intercapillary ball resulting from the transformation of obliterated capillaries. Within the ball are a few shadowy outlines of capillaries. Zenker's fixation, periodic acid-leukofuchsin and Masson's trichrome stains.

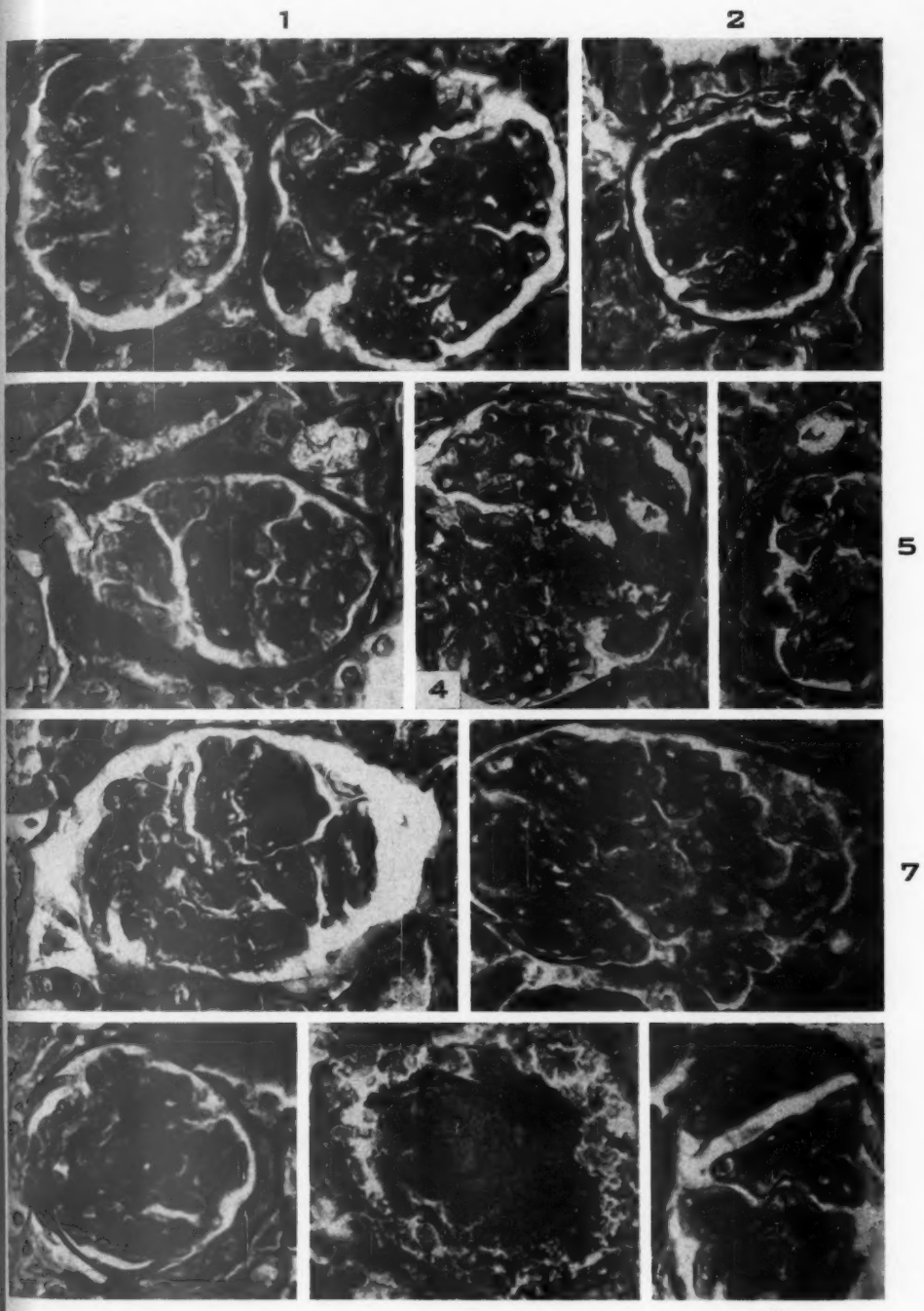
FIG. 7. N 290–50. Same specimen as Figure 1. The reactivity of endothelial cells is indicated. Some capillaries are denuded of reticulin and show irregular, annular fibers.

FIG. 8. N 290–50. Same specimen as Figure 1. Thickening of the capsular membrane upon which rests a row of hyperplastic and hypertrophic capsular cells. They are attached to the glomerular tuft by tiny fibrils. In the remainder of the corpuscle only two narrowed lumina are demonstrable.

FIG. 9. N 290–50. Same specimen as Figure 1. The capillary skein is replaced by an amorphous mass. In the capsular space are extravasated red blood cells.

FIG. 10. N 290–50. Same specimen as Figure 1. Part of the capillary lobe consisting of a group of obliterated capillaries which has detached itself from the other lobes and has become a patch attached to the capsular wall.





Mann, Goddard, and Adams Renal Lesions in Experimental Diabetes

Plate 132

All figures drawn to scale indicated in Figure 11.

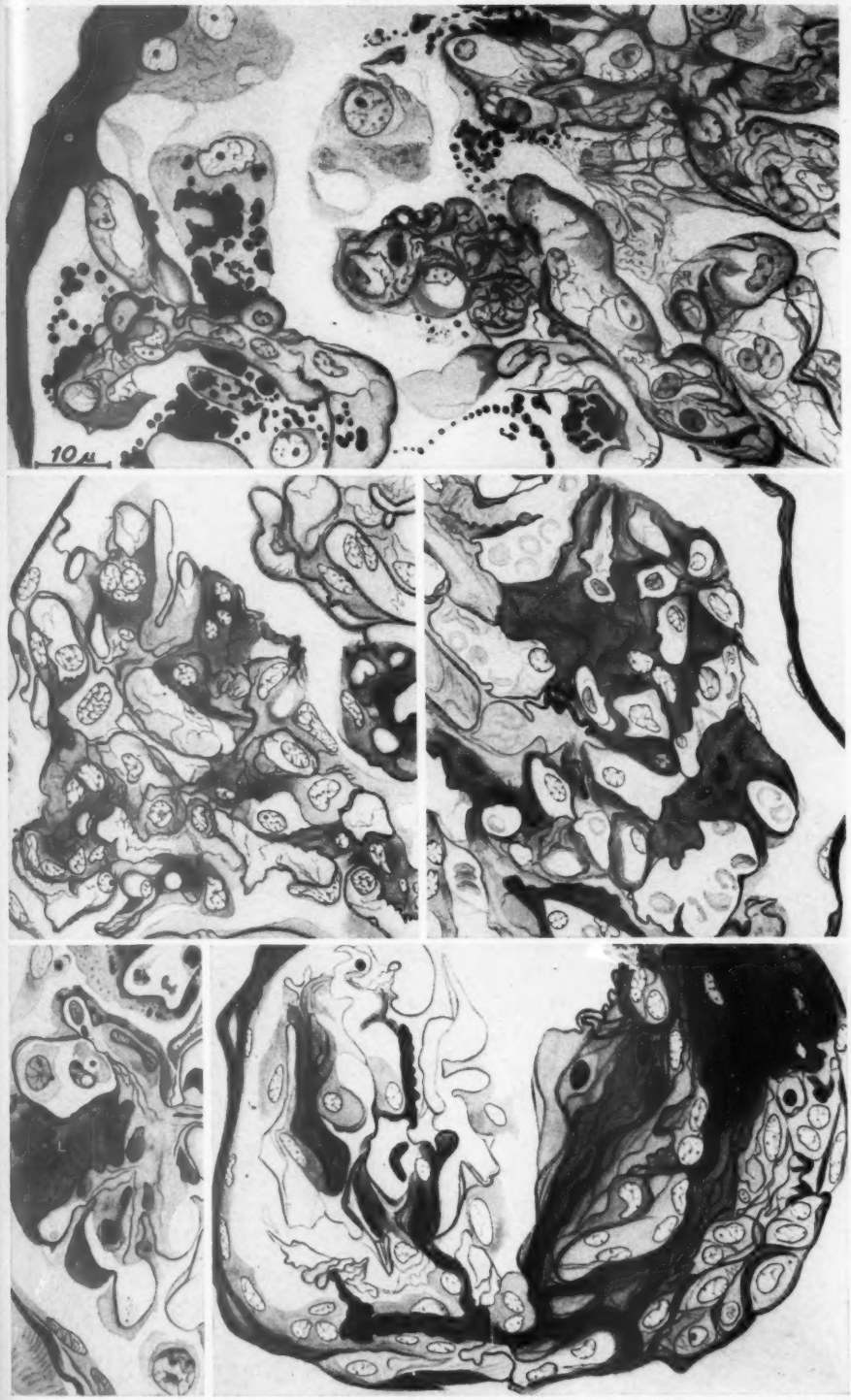
Fig. 11. N 19–50. Same specimen as Figure 4. At left, part of a thickened Bowman's capsule with heavy collagenous bundles. Above, two "serosal" cells are appended to it and below these a capillary loop is attached to the capsule. The lumina of capillary loops below are obliterated by fused reticulin fibers (intraluminal growth). Around the capillaries are irregular clumps and globules of a fuchsinophilic material (represented here in black). By coalescence this material forms intercapillary masses. In the center, an area of disintegrated capillaries which show whorled, intraluminal, and periparietal fibrils. This is an early stage of a fibrillar or reticular ball. In the remainder of the figure may be seen heavy reticulin fibers outlining capillaries, double contours of reticulin fibers, erosion of capillary walls, intraluminal growth of reticular fibrils, and accentuation of the mesangium. This figure represents a third of a glomerulus.

Fig. 12. N 293–50. Duration, 11 months; diet, P–10; severity, 5.3 gm. of glucose per 24 hours. Obstructed and bloodless capillaries are on the left with interstices filled with a dark-staining mass of reticulin matrix pervaded by delicate fibrils. Hyperplasia and hypertrophy of endothelial and epithelial cells. This is an early stage of an intercapillary fibrillar ball. Zenker's fixation, periodic acid-leukofuchsin and Weigert's iron hematoxylin stains.

Fig. 13. N 295–50. Duration, 8 months; chow diet; severity, 2.9 gm. of glucose per 24 hours. This is similar to Figure 12, except for some patent capillaries containing red cells and more extensive and denser intercapillary masses which contain pyknotic epithelial nuclei and fewer reticulin fibrils. In the center a few reticulin bundles have undergone collagenization (wavy; darker shade). Zenker's fixation, periodic acid-leukofuchsin and Masson's trichrome stains.

Fig. 14. N 319–48. Duration, 5 months; diet, P–40; severity, 1.8 gm. of glucose per 24 hours. At the left is a granular patch of fuchsinophilic material containing partially hyalinized globules. The mesangium is accentuated. The endothelial nuclei are pyknotic. Zenker's fixation and periodic acid-leukofuchsin stain.

Fig. 15. N 319–48. Same specimen as Figure 14. About four-fifths of a two-lobed glomerulus is represented. On the left are the remnants of collapsed capillary loops. Endothelial cells are absent except for pyknotic nuclei. Epithelial cells are enlarged. The right lobe illustrates a later state of ball formation. The capillary contours are lost. Bundles of dense reticulin material assume a "scarf-like" course. At the extreme right there is a group of presumably endothelial cells, arranged in a tissue-culture-like fashion. At the upper right-hand corner a ball of piled-up, almost concentric reticulin fibers and bundles is formed (not yet hyalinized).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TRANSFORMATION OF THE HEPATIC VASCULATURE OF RATS FOLLOWING PROTRACTED EXPERIMENTAL POISONING WITH CARBON TETRACHLORIDE

Its Possible Relation to the Formation of Urate Calculi in the Urinary Tract *

H. Ungar, M.D.

(From the Department of Pathology of the Hebrew University-Hadassah Medical School, Jerusalem, Israel)

In a previous article,[1] urate calculi were reported in the bladders of white rats following treatment with carbon tetrachloride over a period of 4 to 6 months. No calculi were discovered until the later stages of the experiment, when extensive hepatic fibrosis had developed. In the discussion of the relation of uric acid excretion in mammals to interference with the uricolytic function of the liver, it was suggested that increased excretion of urates in advanced cirrhosis in rats may be a result of the formation of intrahepatic porto-hepatovenous shunts which may produce physiologically the effects of an Eck's fistula. Following the latter, uric acid sedimentation was reported by an earlier author.[2] The presence of venous shunts has been observed also in human cirrhosis with the aid of injection methods.[3,4] Wakim and Mann [5] examined cirrhotic livers in living white rats with the quartz-rod illumination technic and found that "The vascularization of the regenerated regions of hepatic parenchyma is mainly arterial," a finding which is comparable to that in human livers with Laennec's cirrhosis.

I repeated my previous experiment [1] in order to study the vascular changes in the liver and to determine their rôle in diminishing uricolysis indicated by the formation of urate calculi.

Material and Methods

Forty-five male rats each received fifty subcutaneous injections of carbon tetrachloride, in doses of 0.1 cc. per 100 gm. of body weight twice weekly. The drug was diluted with equal amounts of liquid paraffin in the first thirty injections. The animals were sacrified 40 to 60 days after treatment was terminated.

Diet

The rats were fed on a diet of wheat grain, skim milk, and tap water *ad libitum*. Following the last dose of carbon tetrachloride the animals were divided into two groups. Series 1 (25 rats) continued to receive

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the same diet. In series 2 (20 rats), milk was omitted for the rest of the experimental period since the same group of animals was also to be used for a study of the rôle of nutritional deficiency in the formation of urate calculi in the urinary tract.

At the beginning of the experiment the animals weighed from 40 to 50 gm., and when treatment with carbon tetrachloride ceased their weights ranged between 200 and 240 gm. During the period of deficient diet lasting from 40 to 60 days, the animals lost from 20 to 40 gm. in weight.

Methods of Examination

The animals were sacrificed by bleeding. The vena cava was ligated in the thoracic cavity and was widely opened in the abdomen. The liver was flushed through the portal vein with warm physiologic saline solution containing 1 per cent sodium nitrite. This was followed by the injection of a mixture of three parts of ox serum with two parts of India ink as recommended by Hamburger.[6] The injection was performed easily under pressure of 15 to 20 mm. of Hg. It was continued until the surface of the liver became intensely black, which occurred as a rule within a few seconds both in normal control rats and in many of the experimental animals. The flow of fluid from the vena cava frequently began before blackening of the hepatic surface was completed.

In series 1, initial trials were made with injection of the ink mixture following flushing of the vein with a solution of physiologic saline without the addition of nitrite. This method gave varying results, and on several occasions the injection was successful only with the use of higher pressures (up to 50 to 60 mm. of Hg). In several animals vascular resistance to injection was so great that it could not be overcome without causing extravasation. This also happened after filtration of the ink mixture. Possible causes of this phenomenon will be discussed.

Livers were fixed in Bouin's fluid. Histologic sections were cut from two different lobes in each case and were stained with hematoxylin and eosin and with van Gieson's stain. In special instances, the Prussian blue reaction for hemosiderin, Heidenhain's azan stain, and Foot's method for the impregnation of reticulum fibers were applied.

Contents of the urinary bladder were examined and the kidneys were preserved for histologic examination.

Experimental Results

The amounts of fibrosis found on microscopic examination were not in accord with the classification of macroscopic findings used in the

previous paper.[1] The findings were classified into four types of lesions, as shown in Table I. No differences were found macroscopically and microscopically between animals on complete diet and those which were kept on deficient diet for some time before termination of the experiments.

Type 1 Lesion. Twelve rats of series 1 and 6 rats of series 2 showed lesions classified as type 1. The liver had a smooth or slightly uneven surface with flat nodules, especially on the lower surfaces. Microscopically, the lobules were found to be completely transformed and delimited by discrete septa of collagen tissue (Fig. 1). The liver cells were about normal in size. Binucleated cells were rare. The connective tissue included groups of macrophages containing hemosiderin and undilated veins which frequently lay parallel to the bile ducts. Injection with ink was successful in 16 animals. In these, all veins within the connective tissue were completely injected. Injection of the sinusoids was almost complete, and no lobules were entirely free of the injected ink.

No urate calculi were observed in the urinary bladder, but in one rat of series 2 calcium phosphate calculi were present.

Type 2 Lesion. Four rats in series 1 and 5 rats in series 2 showed lesions of the second type. The liver was normal in size or slightly enlarged. The surface was coarsely nodular, and some areas were covered with flat, raised nodules of varying size (coarsely nodular hyperplasia). The microscopic appearance was not very different from that of the type 1 lesion, except that the connective tissue trabeculae were slightly more prominent and contained proliferated bile ducts which assumed an adenoma-like formation in some areas (Fig. 2). Moreover, several large, prominent nodules of parenchyma were distinguished by uniform cells having a slightly basophilic cytoplasm (nodular regeneration).

Injection was unsuccessful in 3 rats for technical reasons. In 4 of the remaining 6 animals the regenerative nodules were not injected, but in the intermediate parenchyma fully injected sinusoids were present (Fig. 3). The sinusoids in the liver of the remaining animal were almost completely injected, and its connective tissue contained numerous distended, injected veins lying in groups and presenting a varicose appearance (Fig. 4).

Urate calculi were found in the bladder of this animal as well as in another in which injection was not completed owing to air embolism. The bladder of an animal belonging to series 2 contained calculi composed of calcium phosphate.

Type 3 Lesion. Five rats in series 1, and 3 rats in series 2 showed

lesions classified as type 3. The gross aspect of their livers was that of finely nodular cirrhosis; in addition, one liver contained large, soft, regenerative nodules. Histologic examination revealed pseudo-lobules, predominantly of small diameter. The liver cells were of fairly uniform size and contained only a single nucleus. The appearance of the cytoplasm varied, and was rather uniformly eosinophilic or obscured with coarse basophilic granulations. Discrete vacuolization was seen in a few areas. The connective tissue formed delicate strands or moderately wide trabeculae which sometimes were confluent in scar-like patches (Fig. 5). Moderate infiltration of the stroma with small lymphoid cells was observed, as well as scattered plasma cells and accumulations of macrophages containing hemosiderin granules. In many areas bile ducts were seen in increased number. The connective tissue throughout the liver contained veins filled with injection mass and varying in caliber, but usually wider than normal portal ramifications. In certain areas veins were surrounded by sparse connective tissue traversing groups of liver cells without clearly defining pseudo-lobules. The injection mass filled almost all of the sinusoids. In one liver there were isolated, circumscribed nodules which were entirely uninjected.

No calculi were found in the urinary bladder.

Type 4 Lesion. One rat in series 1, and 3 in series 2 showed lesions classified as type 4. Coarsely nodular cirrhosis with fibrosis as well as marked deformation of individual lobes and clearly defined differences between the lobes of a single liver were observed macroscopically; several lobes were considerably contracted and of firm texture, their surfaces being diffusely granulated and dull yellowish brown. The liver of the rat belonging to series 1 was of firm texture and fairly large nodules were seen on its surface, but these were not so large as those classified as type 2. No deformation of the lobes was seen in this animal (Fig. 6).

The histologic picture was identical in every part of the organs. There was extensive destruction of the normal architecture. The parenchyma was composed of pseudo-lobules within a stroma of abundant connective tissue containing numerous proliferated bile ducts and very sparse accumulations of lymphocytes. The pseudo-lobules were composed of liver cords of normal width. The cells were of uniform appearance and fatty change was seen in limited portions of scattered lobules. After injection with India ink the injection mass was found in thin-walled blood vessels as well as in large, rounded cavities. Only

a few pseudo-lobules near the periphery of the hepatic lobe were completely injected; all other lobules were entirely free of injection mass or contained one or two fairly large injected vessels (about the caliber of central veins) showing a few ramifications, but no filling of the sinusoids was seen (Fig. 7).

In sections from the atrophic lobe of liver (Fig. 8) the changes were similar to those previously described. There was excessive overdevelopment of the fibrous stroma. The pseudo-lobules were not always clearly defined, but were subdivided by connective tissue until small groups of liver cells or even single, isolated cells were seen to be surrounded by fibrotic tissue. Many of these cells contained two nuclei which occasionally were small and pyknotic.

Comment

For the study of the venous hepatic circulation by the injection method, I have chosen a fluid of standard composition and freely flowing at room temperature, which was filtered and injected under measured pressures. With these precautions I found that with freshly killed animals it was sometimes impossible to force the fluid beyond the portal ramifications with pressures as high as 60 to 80 mm. of Hg, when extravasation invariably occurred. The same result was observed when physiologic saline solution was injected, but not when animals found dead and rigid in the cage were injected.

A number of authors have remarked upon difficulties in filling the hepatic sinusoids through the portal veins. Ashburn, Endicott, Daft, and Lillie,[7] in a study of the venous circulation in the liver of rats with nutritional or carbon tetrachloride cirrhosis, believed these difficulties to be due to agglutination of carbon particles. Previously, Olds and Stafford [8] had stated that the main obstacle to a uniform filling of the portal ramifications with pressures up to 40 mm. of Hg may be caused by the collapse of the sinusoids after the inflow of arterial blood and the back pressure in the hepatic veins had ceased. They rejected the view of Gilbert and Villaret [9] who maintained that the difficulty in obtaining complete injection of the hepatic lobules by the portal route might be the result of a "vital reaction" of liver cells. This opinion, however, was supported by Bauer, Dale, Poulsson, and Richards [10] on the basis of physiologic studies of the circulation in the isolated liver. These authors concluded that "It is impossible, under the best conditions, to perform an artificial perfusion without some injury to the liver cells." In their view, histamine-like substances are

thereby liberated and these indirectly increase resistance to perfusion.

In view of these observations, in the present study perfusion with 1 per cent solution of sodium nitrite in physiologic saline preceded the injection of the ink-serum mixture and resulted in almost immediate complete filling of all sinusoids and veins in normal rats, under pressures which were only slightly above the physiologic limit. While the dilatation of hepatic blood vessels resulting from the use of sodium nitrite may not reflect natural conditions, it nevertheless yields a "basal tonus" which permits comparison of the vascular transformations in different rats without the interference of otherwise uncontrollable factors due to possible supravital changes.

At the conclusion of the experiments the hepatic architecture of all the test animals was thoroughly changed by the formation of pseudolobules such as would be expected following repeated destruction and regeneration of the hepatic epithelium. Despite the identical treatment of all animals, considerable variation in the degree of regeneration and in the amount of fibrosis were observed. The incidence of the different changes and their association with the presence of urate calculi in the bladder may be seen from Table I.

The injection of the portal vein with India ink led to varying results in accordance with the different types of hepatic lesions. The venous channels within the connective tissue areas were completely and equally injected in all animals, but complete filling of the sinusoids was obtained only in livers with lesions of types 1 and 3. In livers with severe fibrosis (type 4), the hepatic sinusoids appeared to be almost entirely disconnected from the portal flow.

The results were not as uniform in rats with regenerative nodular hyperplasia of the liver (type 2 lesion), in which varying degrees of sinusoidal injection were observed. In these livers regenerative nodules remained uninjected while the sinusoids of the internodular parenchyma were filled entirely with the injection mass. In addition, in several livers the increased interstitial connective tissue contained numerous wide, injected veins which lay close together in varicose formation, a picture which occasionally has been observed in hypertrophic cirrhosis in man.[11]

Urate calculi never occurred when the portal blood flow to the liver parenchyma was intact. Their presence was always associated with severance of lobules from the portal circulation in animals with coarsely nodular cirrhosis following the development of porto-systemic venous shunts. In livers showing the lesion designated as type 2, the

pseudo-lobules were only in part disconnected from the portal circulation, but in the absence of visible damage to the liver cells, this partial deviation of blood may be considered sufficient to explain the excretion of urates in the urine as manifested by the formation of calculi in 2 of 8 rats in this group.

While the findings previously described appear consistent with the original assumption that the excretion of uric acid may be due to

TABLE I

Lesions of the Liver and Urinary Bladder of Rats 40 to 60 Days after Administration of Carbon Tetrachloride Was Discontinued

Type of lesion	Normal diet		Deficient diet after cessation of treatment with CCl_4	
	No. of animals	Urate calculi	No. of animals	Urate calculi
1. Transformation of liver; no fibrosis	12		6	
2. Coarsely nodular hyperplasia; moderate increase of fibrous tissue	4		5	2
3. Finely nodular cirrhosis; moderate increase of fibrous tissue	5		3	
4. Coarsely nodular cirrhosis; marked fibrosis	1	1	3	3
Animals dying during the course of the experiment without signs of cirrhosis	3		3	
Total number of animals	25	1	20	5

elimination of uricolysis following deviation of the portal blood from the parenchyma, which is comparable to the similar disturbance observed in animals with Eck's fistula, they cannot exclude the influence on the formation of calculi of additional general metabolic factors and/or local lesions in the urinary tract which require further study.

My findings require further interpretation with regard to another problem connected with experimental cirrhosis in rats. Hepatic damage following carbon tetrachloride poisoning has been shown by a number of authors to be reversible as long as no abundant fibrous tissue was formed. Cameron and Karunaratne [12] suggested that even fully developed cirrhosis following carbon tetrachloride treatment may regress within about 4 weeks if the rats receive no more than forty injections of carbon tetrachloride in the dose employed in the present study.

They postulated that with more extended treatment, irreversible cirrhosis of the coarsely nodular type will invariably result; and they maintained that reversal of fibrosis depends on an inherent regenerative capacity of the hepatic epithelium, which is impaired by prolonged poisoning. In the present experiments, treatment was continued for a longer period than in those of Cameron and Karunaratne, but nevertheless 1 to 2 months after cessation of treatment only 4 of 39 animals had coarsely nodular cirrhosis. Our results cannot determine whether the remaining 35 animals had reached the end-stage of reversible cirrhosis or whether, for unknown reasons, fibrosis had never progressed in them to a noteworthy degree simultaneously with the reconstruction of the parenchyma.

Assuming that our results are consistent with "reversible cirrhosis," persistence of fibrosis appears to be less dependent on the intrinsic regenerative capacity (which seemed to be uninfluenced by even more continued poisoning, as judged by the abundant regenerative nodules) than on an intact supply of portal blood to the parenchyma. The latter view has been supported by Morrione [13] who demonstrated irreversible cirrhosis in rats in which Eck's fistula was performed after treatment with carbon tetrachloride was discontinued.

Summary

In previous experiments, urate calculi were shown to occur in association with coarsely nodular cirrhosis of the liver in white rats, following protracted treatment with carbon tetrachloride.

The experiments were repeated and the livers were studied histologically following injection of the portal circulation with India ink.

An association was observed between the occurrence of urate calculi and severance of the hepatic parenchyma from the portal blood supply, which was held to be a result of the formation of intrahepatic portosystemic venous shunts.

The results of these experiments have certain implications for an understanding of the elimination of the uricolytic function of the liver and the "reversal" of experimental cirrhosis in rats.

I am indebted to Professor E. Wertheimer and Dr. W. Herz for the chemical analysis of calculi.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

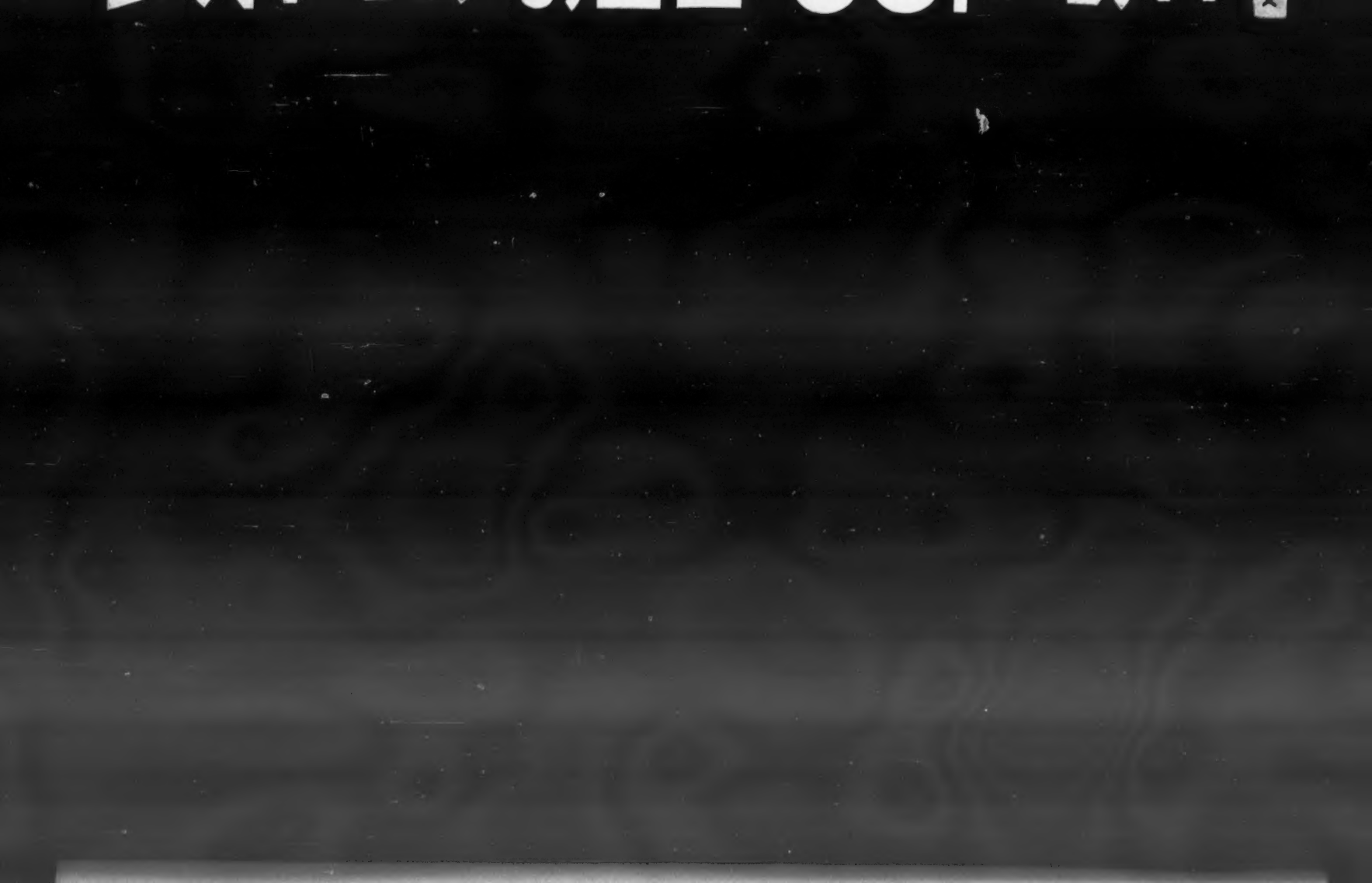
PLATE 133

FIG. 1. Smooth liver, 6 weeks after treatment with carbon tetrachloride was discontinued. Transformation of acini, but no fibrosis (type 1 lesion). Sinusoids completely injected. × 105.

FIG. 2. Coarsely nodular hyperplasia of liver; moderate fibrosis (type 2 lesion). Of note is the adenoma-like proliferation of bile ducts. × 105.

FIG. 3. Regenerative nodular hyperplasia (type 2 lesion). Injection of sinusoids only in internodular parenchyma, but not in regenerative nodules. × 115.

FIG. 4. Same lesion as in Figures 2 and 3, showing varicosity-like accumulation of newly formed venous lumina. × 105.



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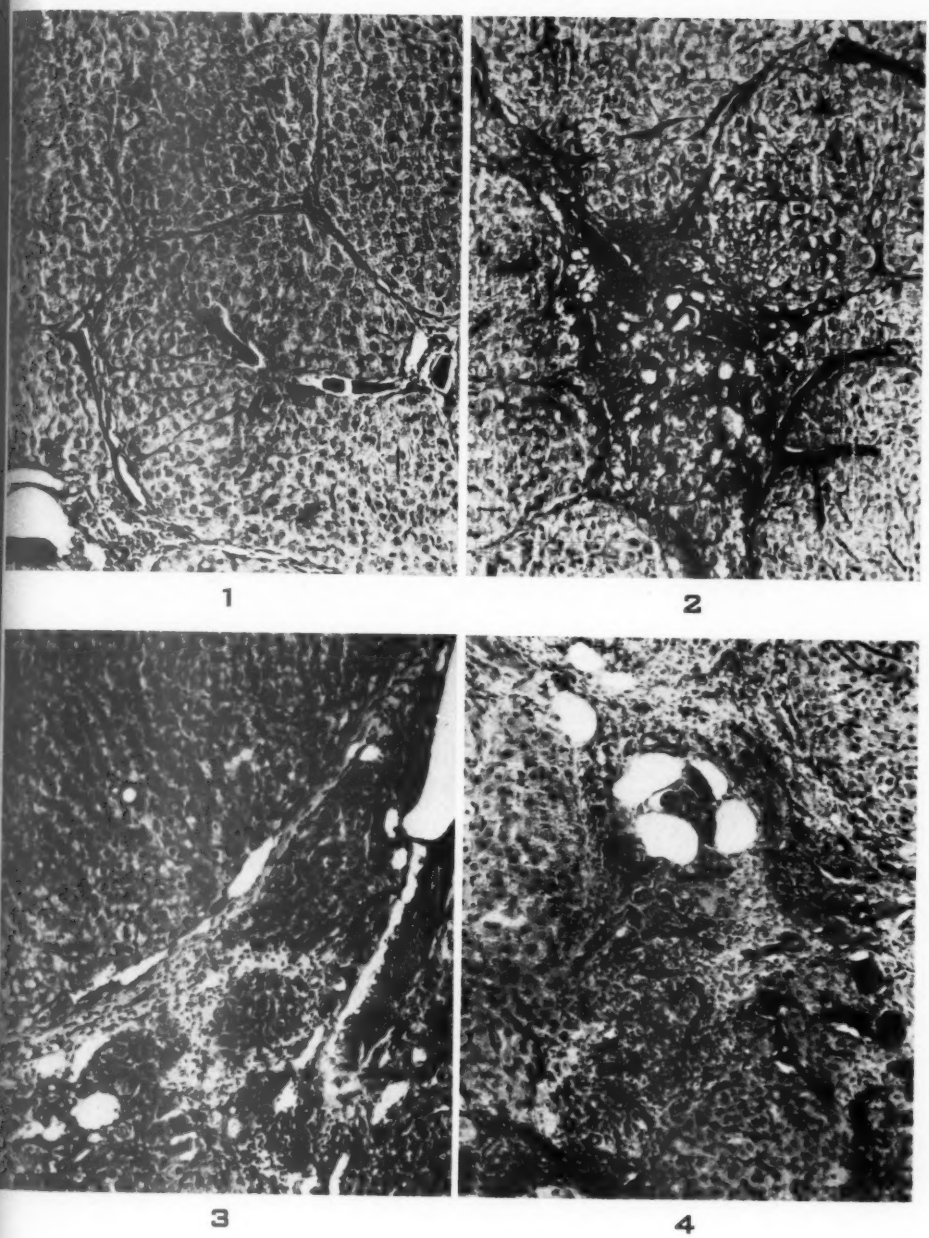


Ungar

Hepatic Vasculature Following Carbon Tetrachloride

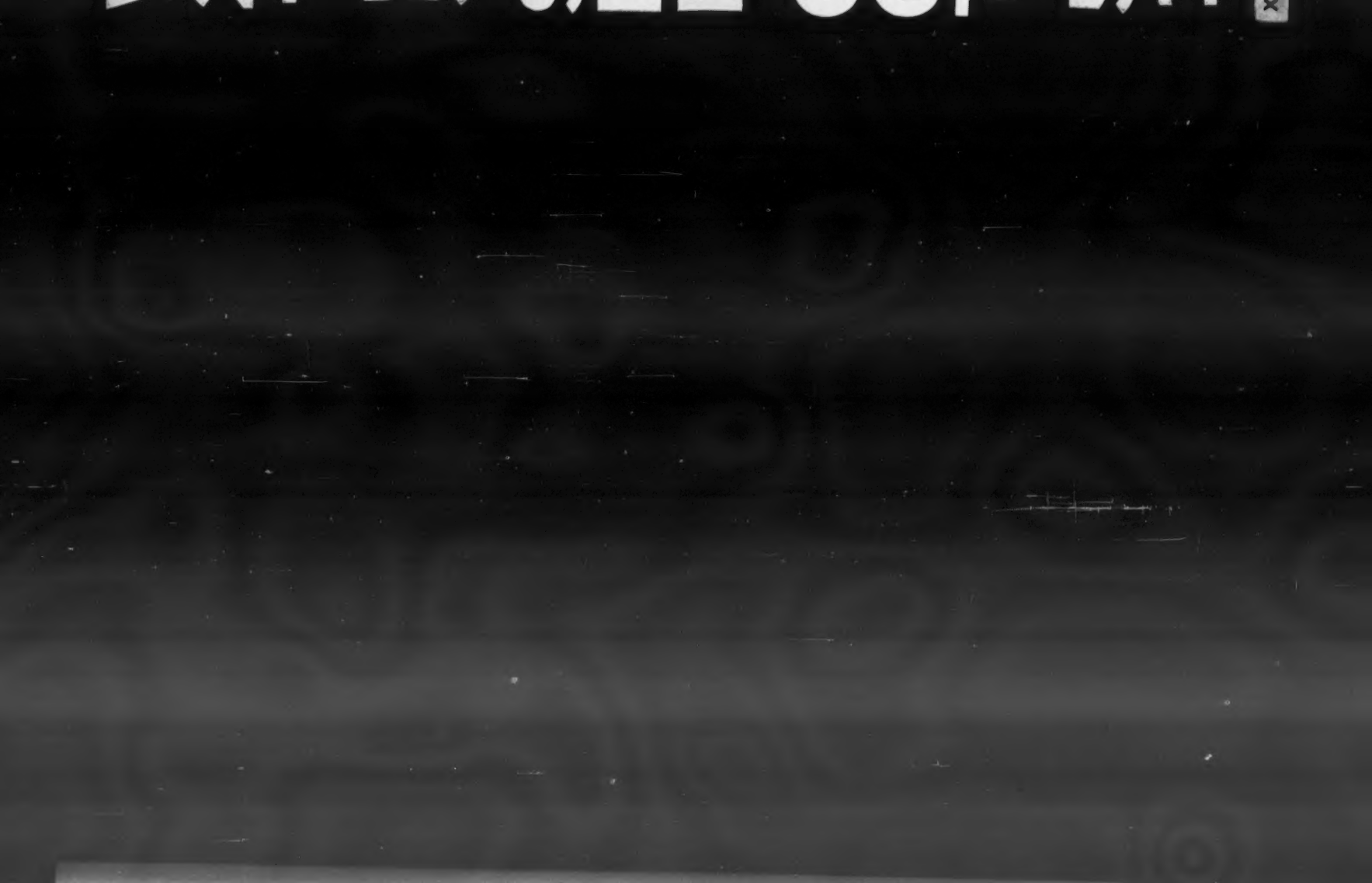
PLATE 134

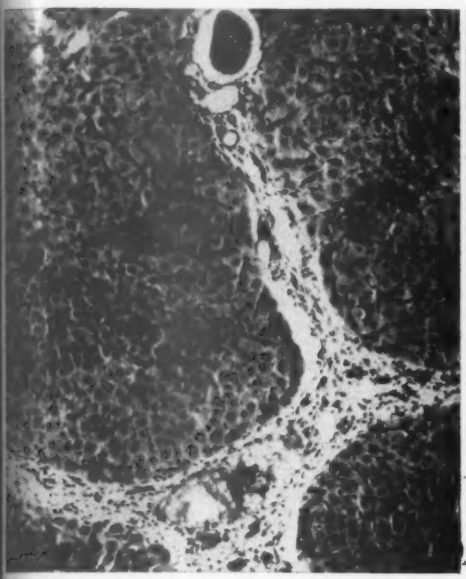
FIG. 5. Finely nodular cirrhosis. Complete injection of sinusoids. × 115.

FIG. 6. Injected liver with coarsely nodular cirrhosis (about 1½ times the natural size). The black nodules are completely injected with ink.

FIG. 7. Coarsely nodular cirrhosis. The fibrotic tissue contains varicosity-like veins. No injection fluid has reached the sinusoids. × 95.

FIG. 8. Coarsely nodular cirrhosis with extensive fibrosis. The sinusoids of the pseudo-lobules remained free of injection fluid. × 38.





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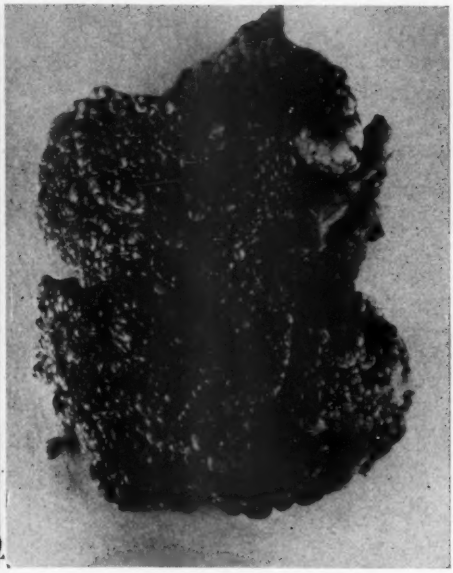

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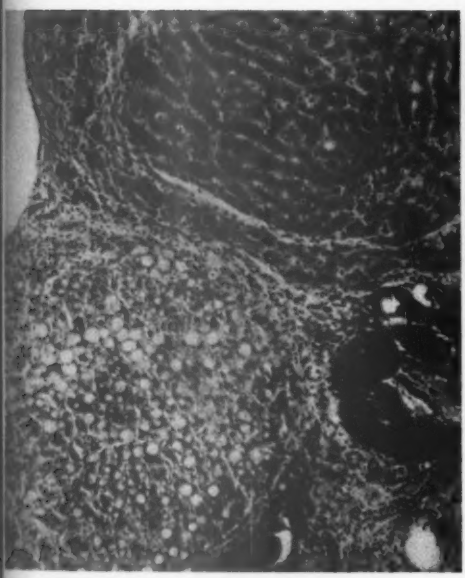

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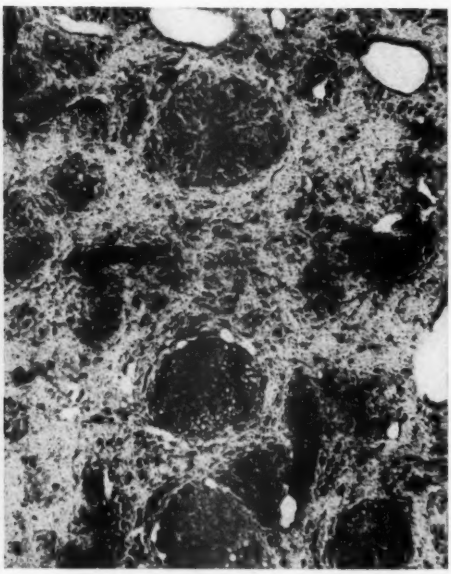

8

Ungar Hepatic Vasculature Following Carbon Tetrachloride

SENECIO POISONING EXHIBITING AS CHIARI'S SYNDROME
A Report on Twelve Cases *

G. Selzer, M.B., Ch.B., and R. G. F. Parker, M.B., B.Chir., D.P.H.
(From the Department of Pathology, University of Cape Town, Cape Town, South Africa)

The ingestion of certain species of senecio (ragwort) has been recognized in several countries, including South Africa, as a cause of poisoning in horses and cattle. To our knowledge, however, the only published information on senecio poisoning in man is that contained in a report by Willmot and Robertson (1920) who studied a few cases of human poisoning by bread containing seeds and leaves of *Senecio burchelli* and *Senecio ilicifolius*.

In the course of work on dietary hepatic necrosis we studied a number of patients who had been diagnosed as cases of senecio poisoning. We realized that this diagnosis had not been fully established, for although all except 2 patients gave a history of having eaten bread made from imperfectly winnowed wheat and in certain cases this was stated to be "contaminated," in no case was an attempt made to confirm the presence of senecio in the flour. Nevertheless, there is much evidence, both epidemiologic and experimental, that the etiologic agent in these cases was in fact a species of senecio which grows as a weed in the wheat in certain districts of South Africa, and which, with imperfect winnowing, may contaminate the flour made from such wheat.

The clinical features of 12 cases of senecio poisoning seen in Cape Town and necropsy findings on the 6 patients who died will be described, and our reasons for considering these cases to be examples of Chiari's syndrome will be given. Chiari's syndrome is characterized by the rapid onset of ascites, hepatomegaly, nausea, and vomiting. In the cases described by Chiari (1899) this was the result of a primary thickening of the intima of the hepatic veins which occurred independently of changes in the surrounding tissue.

Report of Cases

Case 1

C. J. S., a European male, 43 years old, was admitted to the Somerset Hospital, Cape Town, from Citrusdal on June 2, 1931, and left the hospital on June 17, 1931. Four weeks previously he and the rest of his family had had "colds." The symptoms were a dry irritating feeling in the chest and headache. There was no nausea, no vomiting, and no bowel disturbance. Eight days later he complained of severe abdominal pain and his abdomen began to swell. This swelling increased progressively and later his feet, too, became swollen. Two brothers and two female relatives who had "colds" at the same time recovered completely. Three brothers, however, de-

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veloped severe abdominal pain, swelling of the abdomen, and epistaxis. Their diet had consisted of pork, potatoes, beans, pumpkin, and bread, and the whole family had eaten the same food prepared in the same kitchen. None had eaten any unusual food such as mushrooms. It was subsequently ascertained that their bread was baked from flour obtained from a small neighboring mill and that this flour was made from imperfectly winnowed wheat. The patient stated that similar symptoms had been observed in animals in the district. Thirteen mules had developed swelling of the abdomen and had died. The disease had occurred also in sheep, some of which recovered after abdominal paracentesis.

On admission the patient was obviously ill. He had severe generalized abdominal pain and a firm liver enlarged to 3 fingersbreadth below the right costal margin. There was no splenomegaly, no jaundice, and no edema. There was slight pyrexia to 99° F.

The urine contained 1 plus urobilin, 1 plus urobilinogen, but no albumin or blood, and the specific gravity was 1.020. The leukocyte count was 6,400 per cmm. The direct van den Bergh reaction was negative and the indirect measured 0.8 mg. of bilirubin per 100 ml. of blood. The serum albumin was 2.3 per cent and the serum globulin, 3.2 per cent. The ascitic fluid was clear and yellow and contained 3.5 gm. of protein. A levulose tolerance test showed slight impairment of hepatic function.

Paracentesis was performed and salyrgan, ammonium chloride, and glucose were administered. The patient improved considerably but refused to stay in the hospital after his brother (case 2) died.

Case 2

H. P. J. S., a European male, 28 years of age, and brother of the patient reported as case 1, was admitted to the Somerset Hospital on June 2, 1931, and died on June 17, 1931. His illness commenced at the same time as his brother's and the symptoms were identical except that he had frequent epistaxis. On admission he was very ill, with severe abdominal pain. He had marked ascites, but no jaundice or edema and no hepatomegaly or splenomegaly.

The urine contained 2 plus urobilin, and 2 plus urobilinogen, no bilirubin, and no albumin; the specific gravity was 1.020. The leukocyte count was 7,400 per cmm., and the differential count was normal. The serum van den Bergh direct reaction was negative and the indirect reaction measured 2.2 units. The serum albumin was 3.5 per cent; serum globulin, 1.3 per cent; blood sugar, 76 mg. per 100 ml. The levulose tolerance test showed definite impairment of hepatic function, the maximum rise being 40 mg. per 100 ml.

The patient's abdomen was tapped twice, over 10 pints (5.5 l.) of fluid being withdrawn on each occasion. His liver was then found to be 1 inch (2.5 cm.) above the costal margin. He was apyrexial throughout his illness except for an occasional rise of temperature to 99° F. His pulse rate varied from 80 to 120 beats per minute. He developed slight epistaxis and slight bleeding from the gums. On June 16 he became comatose and cyanotic, and died on the following day.

The necropsy record was not available, but a portion of the liver was preserved and this showed a wrinkled capsule, and a cut surface with the appearance of intense passive congestion of varying degree in different parts of the organ. Some of the large hepatic veins was occluded by red thrombi and a similar lesion was present in one portal vein (Fig. 1).

Histologically, the liver showed a lesion which at first sight appeared to be an extensive hemorrhagic zonal necrosis, varying in extent in

different areas but involving on an average about one-half of each lobule. The central and sublobular veins were surrounded by sheets of red blood corpuscles in which only occasional isolated liver cells and Kupffer cells survived. In spite of the hemorrhagic appearance, however, reticulum stains revealed that the reticular framework in these zones was almost intact. The Prussian blue reaction showed no increase in hemosiderin. There was no inflammatory cell infiltration and no recent necrosis of liver cells. The only sign of parenchymal regeneration was the presence of occasional, large, hyperchromatic nuclei. The fibrous tissue of the portal tracts was not increased and there was no bile duct proliferation. In some central zones the reticulum was thickened but the change was not sufficiently marked for it to be called fibrosis.

The changes in the blood vessels were of great interest. The hepatic arteries and, with one exception, the portal veins were normal. The detailed structure of the central and sublobular veins was often difficult to visualize in hematoxylin and eosin sections, but in many cases there appeared to be necrosis of the vessel wall with loss of nuclear staining and infiltration with red blood corpuscles. The lumina of the great majority of both necrotic and non-necrotic vessels were partly and often wholly occluded by fine fibrils which stained poorly with hematoxylin and eosin but well with Foot and Day's reticulum stain (Figs. 3 and 6) and which sometimes gave the reaction for collagen with van Gieson's stain. Most of the scanty nuclei present were fibrocytic rather than fibroblastic in type. The lumina of the partly occluded vessels were usually single but occasionally multiple. Of the two large hepatic veins in the section, one about 2 mm. in diameter appeared normal, while the other, 9 mm. in diameter, contained a red thrombus showing early organization. The latter showed no inflammatory changes in its wall.

Case 3

H. P. T., a European male, 50 years old, was admitted to the Somerset Hospital, Cape Town, from Knysna on April 23, 1935, and discharged on June 29, 1935. Seven weeks before admission he and 3 of his children became ill. The illness was attributed to eating bread made from contaminated flour, though his wife and 2 other children who had eaten the same bread remained well. The initial symptom in all was severe abdominal pain, at first epigastric and later generalized. Two of the children vomited but none had diarrhea. A few days after the onset of the illness all 4 developed ascites and large quantities of fluid were removed by their doctor on several occasions. All 3 children died, two at Knysna and one at Cape Town (case 4).

On examination there was no jaundice nor edema. Ascites was moderate. The liver was smooth, of rubbery consistency, and enlarged almost to the umbilicus. The spleen was not palpable. The other systems were normal.

On May 2, 1935, the urine contained only a faint trace of urobilin. The erythrocyte

count was 4,400,000 per cmm.; hemoglobin, 15 gm. per 100 ml.; leukocyte count, 9,200 per cmm. with a normal differential count. There were slight anisocytosis and poikilocytosis. The van den Bergh direct reaction was negative; serum bilirubin, 0.4 mg. per 100 ml.; icteric index, 5; blood urea, 46 mg. per 100 ml.; blood sugar, 100 mg. per 100 ml.; serum calcium, 7.7 mg. per 100 ml.; plasma cholesterol, 240 mg. per 100 ml. On June 7 the van den Bergh direct reaction was positive; icteric index, 5; serum bilirubin, 0.7 mg. per 100 ml.; blood urea, 41 mg. per 100 ml.; blood sugar, 45 mg. per 100 ml.; serum calcium, 9.8 mg. per 100 ml.; plasma cholesterol, 175 mg. per 100 ml.; serum chloride, 497 mg. per 100 ml. as NaCl; plasma inorganic phosphorus, 5.1 mg. per 100 ml. A levulose tolerance test on June 26 showed slight impairment of liver function; the maximum rise was 35 mg. per 100 ml., and the curve was prolonged. The Wassermann reaction of the blood was negative.

The patient was apyrexial throughout his illness. Fluid was twice removed from the abdomen, 10 and 12 pints (5.5 and 6.6 l.), respectively, being withdrawn. The patient improved on general treatment; he gained weight and was discharged, feeling well, on June 29.

Case 4

A. T., a European female, 10 years of age, was admitted to the Somerset Hospital on April 23, 1935, and died on May 17, 1935. The child became ill at the same time as her father (case 3) and her symptoms were similar to his. During the 7 weeks before admission her abdomen was tapped on several occasions. On admission, she was jaundiced and had marked ascites. The liver was enlarged 4 to 5 fingersbreadth below the right costal margin, and was firm with a sharp edge. The urine contained urobilin but was otherwise normal. Her abdomen was tapped three times while she was hospitalized and on each occasion about 4 pints (2.2 l.) of clear greenish fluid with protein content of 0.45 gm. per 100 ml. were withdrawn. The patient was in the hospital for 3 weeks and throughout this period she was very ill. She was pyrexial during the first and third weeks, her temperature rising to a maximum of 101° F. She developed terminal pleural effusions and remained fully conscious until her death.

Necropsy Findings

At necropsy, the body was emaciated and there was no jaundice. The veins over the right side of the abdomen and thorax were prominent. There was marked ascites. The liver weighed 750 gm., and was tough and flabby with a slightly wrinkled capsule. The cut surface showed a mottled appearance, resembling passive congestion. The veins of the diaphragmatic and falciform ligaments, but not those of the esophagus, were prominent. The spleen weighed 78 gm. The small bowel was thick-walled and sodden, with a thickened, opaque, peritoneal coat, but with no mucosal lesions. There were bilateral pleural effusions with collapse of both lungs.

Histologically, the hepatic lesions resembled those in case 2, from one-fifth to two-thirds of every lobule being affected. Around the central and sublobular veins the liver cells were replaced by red blood corpuscles which appeared fresh and stained normally. Although on the whole well preserved, the reticulum had almost entirely disappeared in a few of the larger lesions, and no excess of hemosiderin was demonstrated. There was no inflammatory cellular infiltration except in the

portal tracts, no evidence of recent necrosis of liver cells, and minimal signs of parenchymal regeneration. Occasional liver cells showed fatty change. There was a slight but definite increase in the portal connective tissue (Fig. 5), some bile duct proliferation, and a lymphocytic infiltration of the portal tracts.

As in case 2, no lesions were evident in the hepatic arteries and portal veins, but few of the central and sublobular hepatic veins were normal. In those areas in which there was destruction of the reticular framework the central veins could not be identified. Elsewhere they showed partial or complete obliteration of their lumina by a tissue which gave the same staining reactions as that seen in case 2. Only occasional vessels were necrotic. The largest hepatic veins in the sections, of a diameter of approximately 1 mm., showed what appeared to be an intimal thickening resulting in a great reduction of the lumen.

Case 5

C. D. V. R., a European male, 13 years old, was admitted to the Somerset Hospital from Knysna on July 10, 1935, and discharged on October 4, 1935. He lived on the farm from which flour had been supplied to the family of the patients described as cases 3 and 4. He had recently eaten bread baked from this flour, and was the only member of his family to become ill. Five weeks before admission he complained of frontal headache and a week later developed epigastric pain unrelated to food. For 1 week he had noted progressive swelling of the abdomen. From the onset of his illness he had suffered from alternating constipation and diarrhea, but had never had any blood or mucus in the stools.

On examination he was thin, had marked ascites, but no jaundice or edema. His liver was enlarged to 4 fingersbreadth below the right costal margin. The other systems were normal.

The urine was normal. The erythrocyte count was 4,400,000 per cmm.; leukocyte count, 10,200 per cmm.; bilirubin, 0.4 mg. per 100 ml.; icteric index, 2.5.

The patient was apyrexial throughout his illness. His abdomen was tapped four times, yielding amounts varying from 8 to 14 pints (4.4 to 7.7 l.). He was treated with salyrgan, glucose, and calcium, and he left the hospital apparently cured.

Case 6

A. S. S. was a European female, 29 years of age, who was admitted to the Somerset Hospital from Knysna on July 23, 1937, and discharged on August 31, 1937. She stated that for about 2 months she had eaten bread made from flour bought from a neighboring farmer and presumably this was not her usual source of supply. For 1 month she had had continuous epigastric pain which was aggravated by meals and which later increased in severity and settled in the right hypochondrium. She vomited on several occasions, had noted slight distention of her abdomen, and was constipated. She developed headaches and feverishness. Two of her children (cases 7 and 8) died while she was in the hospital, and one was very ill. A similar illness occurred in two other families who had eaten the same flour.

On examination, there was no jaundice although the patient said that she had been jaundiced for 1 week. Ascites was present and her liver was just palpable. The other systems were normal.

The erythrocyte count was 4,730,000 per cmm.; hemoglobin, 80 per cent; leukocyte count, 7,200 per cmm. The Wassermann reaction of the blood was negative.

The patient was apyrexial throughout her illness. She gradually improved and was discharged 5 weeks after admission.

Case 7

E. S., a European female, 4 years of age, and child of the patient reported as case 6, was admitted on July 23, 1937, and died on July 28, 1937. She was very ill, with marked ascites and a firm liver enlarged to 3 fingersbreadth below the right costal margin. There was no jaundice and no generalized edema. The urine contained traces of albumin and urobilin. The protein content of the ascitic fluid was 1.6 gm. per 100 ml. The temperature was normal on admission but on the third day it commenced to rise, reaching 103° F. on the day before death.

Necropsy Findings

At necropsy, there was slight edema of the legs, abdominal wall, and retroperitoneal tissues. The veins of the front of the chest were prominent. There was a small quantity of fluid in the pericardial and pleural sacs, and marked ascites. The liver weighed 555 gm. It was flabby with a mottled red and yellow exterior and the appearance of intense passive congestion on section. Several hepatic veins were occluded by red thrombi (Fig. 4). While edema of the stomach and the small intestine was slight, in the large intestine it was very marked and the mucosa was raised in white, cushion-like nodules commencing at the ileocecal junction and becoming progressively less towards the rectum (Fig. 7). The spleen weighed 40 gm., and was normal in appearance. The left kidney was distorted by old pyelonephritic scars. The brain showed suppurative meningitis.

Histologically, the whole liver was involved but the process was more extensive and uniform than in the previous cases. At least one-half of every lobule was affected, and in some lobules there was only a single layer of cells surviving around the portal tracts. Again the lesion was related to central and sublobular hepatic veins, and not to the portal tracts or larger hepatic veins. The appearance of the lesion resembled that of the cases previously described, with survival of reticulum, no excess of hemosiderin, no recent necrosis or evidence of regeneration of liver cells. There were foci of polymorphonuclear neutrophilic cellular infiltration in a few of the affected areas. The liver cells adjacent to the hemorrhagic areas, but not those immediately surrounding the portal tracts, contained vacuoles suggesting a fatty change. In occasional areas fibrin thrombi were seen in sinusoids immediately adjacent to the hemorrhagic zone. There was no fibrosis of the liver.

The hepatic arteries and portal veins were normal. The changes in the central and sublobular veins were essentially similar to those already described (Fig. 2). Occasional veins were necrotic. A few cen-

tral veins appeared unaffected even in markedly hemorrhagic lobules. Hepatic veins measuring from 0.5 to 3 mm. contained red thrombi showing early organization.

The bowel showed very extensive edema of the submucosa and a mild chronic inflammatory reaction in the subserosa. An infarct and an organizing thrombus were found in the lung, but the other pulmonary vessels appeared normal. Chronic pyelonephritis of the left kidney was confirmed.

Case 8

F. S., a European male, 11 years old, and child of the patient reported as case 6, was admitted to the Somerset Hospital on July 23, 1937, and died on August 26, 1937. He became ill 15 days before admission, with diarrhea followed by diffuse epigastric pain. Later he commenced to vomit at irregular intervals after taking food. There was no blood in the vomitus. The most recent symptom was progressive swelling of the abdomen.

The chief findings were marked ascites and a firm, tender liver enlarged to 3 fingersbreadth below the right costal margin. There was no jaundice.

The urine contained urobilin and sugar, and had a specific gravity of 1.028. The erythrocyte count was 4,280,000 per cmm.; hemoglobin, 12.5 gm. per cent; leukocyte count, 9,000 per cmm., with a normal differential count. The serum albumin was 3.2 per cent; serum globulin, 3.5 per cent; icteric index, 9; plasma cholesterol, 90 mg. per 100 ml.; fasting blood sugar, 95 mg. per 100 ml. The levulose tolerance test on August 13 showed a maximum rise to 35 mg. per 100 ml. The Wassermann reaction of the blood was negative.

The temperature usually was normal but on three occasions rose to 100° F. The patient's condition steadily deteriorated and he died 5 weeks after admission to the hospital.

Necropsy Findings

At necropsy, the child was markedly emaciated and there was edema of the legs. The veins of the right thoracic wall were prominent. The abdomen contained a large quantity of clear, yellow fluid and there was an excess of similar fluid in the pleural and pericardial sacs. There was dilatation of the veins along the lateral aspect of the ascending colon, in the ligamentum teres hepatis, along the attachment of the diaphragm on the left side, and at the lower end of the esophagus. The liver was flabby, weighed 1035 gm., and had the appearance of passive congestion. Occasional large hepatic veins were occluded by red thrombi. There was edema and congestion of the stomach and edema of the large intestine, most marked from the ileocecal valve to the middle of the transverse colon. The spleen weighed 75 gm. and appeared normal. The other organs were normal in appearance.

Histologically, the hepatic lesions were diffuse, involving from one-half to almost the whole of every lobule. They were related to central and sublobular veins but not to the portal tracts and larger hepatic veins, and resembled those of the previous cases in showing preserva-

tion of reticular framework except in occasional areas, no excess of hemosiderin, no recent necrosis, no evidence of liver cell regeneration, and no inflammatory cell infiltration. There was marked fatty change in the surviving liver cells at the periphery of the lesion but not in those immediately adjacent to the portal tracts. There was no fibrosis.

The hepatic arteries and portal veins were normal. The central and sublobular veins showed the lesions already described (Fig. 8) except that in the areas where the reticular framework was not demonstrable the central veins could not be identified. All hepatic veins of approximately 1 mm. in diameter, seen in the sections, showed what appeared to be intimal thickening with narrowing of the lumen. The largest vein in the section, measuring 4 mm. in diameter, showed no inflammatory changes in its wall but contained a fairly recent red thrombus showing more organization than in veins of similar size in other cases.

Case 9

M. A. was a colored female, 24 years old, who was admitted to the Groote Schuur Hospital from Knysna on November 26, 1940, and died on December 10, 1940. For 2 months she had suffered from severe colicky pain at the umbilicus, aggravated by food, and from swelling of her abdomen and feet. The abdominal swelling became progressively worse. She had severe headache and was constipated. Three of her children (cases 10 and 11) were suffering from the same illness. On examination she was gravely ill. Massive edema of the back and legs and marked ascites were present, and the liver was enlarged. There were dilated veins in the abdominal wall. Jaundice was absent. The other systems were normal. The patient was apyrexial. Paracentesis was performed on two occasions, 16 and 14 pints (8.8 and 7.7 l.) respectively being removed.

The leukocyte count was 8,700 per cmm.; hemoglobin, 73 per cent; erythrocyte count, 3,730,000 per cmm.; mean corpuscular fragility, 0.34; serum bilirubin, 3.1 mg. per 100 ml.; blood urea, 22 mg. per 100 ml.; plasma cholesterol, 86 mg. per 100 ml. The ascitic fluid was clear and yellow with no clot and with a protein content of 1.1 per cent.

Necropsy Findings

At necropsy, there was edema of the subcutaneous tissues of the thorax and abdomen and marked ascites, 15 pints (8.25 l.) of clear yellowish green fluid being present. There was also clear fluid in both pleural cavities. There was no jaundice.

The liver weighed 850 gm. There was irregular mottling of the serous surface and the appearance of the cut surface resembled that of advanced passive congestion. Multiple sections of the liver failed to reveal any thrombi to the naked eye. The spleen weighed 70 gm. and apparently was not abnormal. The stomach and the small bowel were normal, but there was extreme edema of the colon commencing at the ileocecal junction and becoming progressively less toward the rectum. The kidneys appeared normal. Multiple hemorrhages were present in the vesical mucosa.

Histologically, the hepatic lesions were diffuse, involving one-third to two-thirds of every lobule. They were related to the central, sublobular, and slightly larger hepatic veins, resembling the previous case in showing preservation of the reticulum, no evidence of recent necrosis or of regeneration of liver, and no inflammatory cellular infiltration. Many of the surviving liver cells showed fatty change. There was no fibrosis.

The vascular changes in this case were very marked. The portal veins and hepatic arteries were normal. The central, sublobular, and slightly larger hepatic veins showed to a marked degree the changes described in other cases (Fig. 9).

The large bowel showed marked mucosal and submucosal edema but in addition there was an extensive polymorphonuclear neutrophilic cellular infiltration in the submucosa. Except for foci of hemorrhage in the lungs, the other organs were normal.

Case 10

A. A., a colored male, 7 years of age, was a child of the patient reported as case 9. He was admitted to the Groote Schuur Hospital on November 26, 1940, and discharged on September 9, 1941. He had had swelling of the abdomen and slight swelling of the feet for 1 month and colicky abdominal pain for 1 week.

On examination there was no jaundice. Edema of the abdominal wall, sacrum, and legs was present. There was marked ascites and a smooth, firm, non-tender liver extending to 5 fingersbreadth below the right costal margin. The spleen was not palpable. The other systems were normal.

The van den Bergh reaction was negative; blood urea, 19 mg. per 100 ml.; plasma cholesterol, 71 mg. per 100 ml.; serum albumin, 2.7 per cent. On June 30, 1941, hippuric acid excretion after oral administration of 6 gm. of sodium benzoate was equivalent to 1.13 gm. of benzoic acid. The Wassermann reaction of the blood was negative. The ascitic fluid was pale and yellow, with no clot and with a few lymphocytes and monocytes and a protein content of 1.2 per cent.

On admission 7 pints (3.8 l.) of ascitic fluid were removed, and subsequently paracentesis was performed 23 times. The patient had a slight fever on admission and, for a few days later, a swinging temperature rising to a maximum of 102° F. He was discharged from the hospital apparently well.

Case 11

E. A., a colored female, 5 years of age, and a child of the patient reported as case 9, was admitted to the Groote Schuur Hospital on November 26, 1940, and discharged on December 27, 1940. No clinical history was obtained. On examination there was ascites and the liver was palpable. There was no jaundice and the spleen was not palpable. The urine was normal. The van den Bergh reaction was negative; serum bilirubin, 0.7 mg. per 100 ml.; blood urea, 21 mg. per 100 ml.; plasma cholesterol, 89 mg. per 100 ml. The child was discharged from the hospital apparently well.

Case 12

K. S. was a European male, 58 years old, who was admitted to the Groote Schuur Hospital from the Knysna district on January 22, 1941, and died on February 13, 1941. Three weeks before admission he developed a continuous, gnawing pain in the right hypochondrium, aggravated by food. Four days later he noticed swelling of

the abdomen. He vomited on several occasions and was constipated. Two days before admission his wife had remarked that his eyes were yellow. No other person in his family was affected, but on a neighboring farm three natives developed abdominal swelling and died within 3 weeks. It was stated that they had eaten bread made from wheat contaminated with the senecio plant. On examination there was very slight jaundice and marked ascites, and the liver was enlarged to halfway between the costal margin and the umbilicus.

The serum albumin was 3.2 per cent; serum globulin, 3.6 per cent; van den Bergh reaction, negative; serum bilirubin, 3 mg. per 100 ml.; hippuric acid excretion equivalent to 1 gm. of benzoic acid; urobilin in urine, 2 plus. The ascitic fluid was clear and yellow with a protein content of 1 per cent. The Wassermann reaction of the blood was negative. Sixteen pints (8.8 l.) of fluid were withdrawn from the abdomen and paracentesis was repeated on several occasions. The patient was apyrexial throughout his illness. He steadily deteriorated and died 3 weeks after admission.

Necropsy Findings

At necropsy, there was jaundice but no edema. There was a moderate ascites, the fluid being clear. The liver weighed 1070 gm. and showed extensive passive congestion considerably altered by post-mortem decomposition.

The spleen was firm and red and weighed 250 gm. At the fundus of the stomach were multiple hemorrhagic erosions, possibly post mortem in origin. The small intestine was normal but there was marked edema of the cecum and ascending colon.

Histologically, the liver was uniformly involved, about one-half of each lobule being affected. The lesion was again related to central and sublobular veins. The reticular framework was preserved, there was no evidence of regeneration, no inflammatory cell infiltration, and no excess of hemosiderin. Degenerative changes in the surviving liver tissue could not be assessed because of post-mortem changes. There was no fibrosis. The vascular lesions were confined to the central and sublobular veins and were similar to those described in the previous cases.

Discussion

Clinical Features

The tendency of the disease to occur in households is strikingly evidenced by the fact that 10 of the 12 cases reported in this paper came from three families. Two additional cases occurred in these families, making an incidence of 4 in each family. All of the patients came from districts in which the condition was already well known, and 2 patients (cases 1 and 2) reported that a similar disease had occurred locally among the animals. The relationship of the disease to the consumption of bread made from imperfectly winnowed wheat has been discussed in the introduction to this paper. The patients had com-

plained that the bread had an abnormal taste, some describing it as "musty" and some as bitter.

The most common and most constant symptoms in all patients were abdominal pain and swelling. The former was described as gnawing and occasionally as colicky. It was usually severe, often continued for weeks, and frequently was situated in the epigastrium. A marked, rapidly developing ascites invariably was the most prominent feature and frequently was an early manifestation occurring within the first few days of the illness. It necessitated repeated tapping of the abdomen but eventually disappeared in patients who recovered. The liver usually was enlarged, often markedly. The infrequency of jaundice is noteworthy; it was encountered in only 2 patients, case 12 in whom it was mild and case 4 in whom it was transient. In contrast to the findings in the only other recorded cases of senecio poisoning in man (Willmot and Robertson, 1920), vomiting and diarrhea were not a prominent feature in our cases. Vomiting occurred in only 4 patients and diarrhea in only 2, and in all these symptoms were slight. There was slight edema of the legs in 3 of the patients and in 2 others the edema was more widespread. Four of the patients had bilateral pleural effusions. In none was there a palpable spleen. Only 3 patients showed more than a slight rise of temperature.

The illness was severe and was associated with a high mortality. Six of the 12 patients died, one with a suppurative meningitis. Five left the hospital feeling well and with no ascites, and one left before recovery was complete. Males and females were affected with equal frequency and there was no predilection for any age group.

In those cases in which levulose tolerance and hippuric acid excretion tests were carried out, there was impairment of liver function. The lowered level of serum albumin seen in some patients possibly was related to disturbed liver function or possibly to loss of protein in the ascitic fluid.

Pathologic Findings

In the 5 cases for which necropsy records were available, the significant lesions were confined to the liver, large bowel, and peritoneal cavity, and in a sixth case, in which only the liver was preserved, changes were present in that organ. The liver was enlarged in all except cases 9 and 12, and in these hepatomegaly had been reported on admission. The appearance of the cut surface usually was similar to that of long-standing passive congestion except that the process was less uniform (Figs. 1 and 4). In 3 cases (nos. 2, 7, and 8) thrombi visible to the naked eye were present in some of the hepatic veins. In

4 of the 5 cases for which necropsy records were available, there was marked edema of the large bowel, strikingly confined to this part of the gastro-intestinal tract and so intense that the mucosa was raised into soft, cushion-like nodules (Fig. 7). In the fifth case the small bowel was edematous. In 4 patients, prominent veins were noted in the abdominal and thoracic walls, but further evidence of an anastomotic circulation was not found. Ascites was a striking feature in all. In only one patient, case 12, was there slight splenomegaly (250 gm.).

Histologically, although the duration of the illness had ranged from 5 to 10 weeks, the liver in all cases presented a strikingly similar picture. Almost every liver lobule in the sections examined showed lesions which were situated around the central and sublobular veins and only exceptionally extended to the portal tracts. In these regions the liver cells had almost entirely disappeared and in their place were sheets of red blood cells and Kupffer cells, some of which were rounded and phagocytic. The surviving liver cells in the peripheral zones of the lobules showed fatty change but no evidence of recent hepatic necrosis and little or no regeneration. Some "wear and tear" pigment was present but there was no excessive hemosiderin. Fibrosis was seen in only one case, in which it was portal in distribution and was not marked.

It can be argued that the lesion thus far described could be produced in one of four ways: by hemorrhage from the central or sublobular veins, by centrilobular zonal necrosis, by generalized chronic passive congestion, or by local obstruction to the hepatic circulation (Chiari's syndrome). That the condition is a primary hemorrhagic disease would seem extremely unlikely for the following reasons: (1) The red blood cells were well preserved although the patients had been ill for as long as 10 weeks. (2) There was no local siderosis. (3) There were no signs of repair by granulation tissue or fibrosis. The absence of fibrosis and of liver cell regeneration is as strong an argument against the second hypothesis—that the condition is one of zonal necrosis. There is no support at all, either clinically or pathologically, for the third suggestion of chronic passive congestion of primary cardiac or pulmonary origin. Thus, by exclusion, the evidence points to a lesion in the hepatic veins producing local passive congestion. In every case such a lesion was, indeed, present in the central and sublobular veins. The lumina of these vessels were partly or completely occluded by an open connective tissue with the following staining reactions. Apart from its scanty nuclei, it was seen with difficulty in hematoxylin and eosin sections and no fibrin could be demonstrated by Gram's stain. Its thicker fibers gave the reaction for collagen by van Gieson's method but its extent was best demonstrated by reticulum stains (Figs. 2, 3, 6,

8, and 9). In some vessels occlusion was complete and the lumen when present was minute and often eccentrically placed; occasionally, however, there were multiple lumina.

The tributaries of the hepatic veins of a diameter of from 0.5 to 1 mm. either were normal in the stained sections or showed a change similar to that described above, but with a relatively larger lumen. Of the few larger hepatic veins which appeared in the sections, some contained thrombi showing signs of organization, which in only one case appeared to be of more than a few days' duration. In addition, some of the affected central and sublobular veins were devoid of nuclear staining; their walls were not swollen and no fibrin could be demonstrated in them. It is impossible to avoid describing them as necrotic, but the impression was formed that this loss of nuclei had been a gradual process more akin to degeneration than to ordinary acute necrosis. Inasmuch as these vessels lay in an area of gross circulatory impairment, such a slow progressive degeneration was to be anticipated. It was obvious that this lesion was secondary to an obstructive lesion already present in the smaller vessels. The organizing thrombi in the large hepatic veins were recent and probably also secondary. In at least one case the large veins were free from obstruction. It is true that the number of medium-sized hepatic veins examined was limited, but such lesions as were seen in them were recent.

Of the vascular lesions in the liver, those in the sublobular and central veins would thus appear to be primary. The exact nature of these lesions is very difficult to determine. Either they represent organization and recanalization of a thrombus or proliferation of the intima of the vessel—an endophlebitis. To distinguish between these two processes, especially in vessels of the size of central veins, may be very difficult, as Harrison (1948) recently has emphasized. We do feel that it is not possible, on the histologic evidence alone, to make such a distinction in our material.

From the pathologic aspects, therefore, our cases are examples of Chiari's syndrome (Thompson and Turnbull, 1912; Kelsey and Comfort, 1945; Hirsh and Manchester, 1946). The changes in the parenchyma resemble those in the second case of Thompson and Turnbull in which there was "complete destruction by haemorrhage of the parenchyma save for a narrow zone of cells round the portal systems" and in which there was no fibrosis and no regeneration. Fibrosis was present in Thompson and Turnbull's first case and is noted by Himsworth (1950) as occurring in chronic cases. It was not present to any significant degree in our material. Localized edema of the large intestine was not present in either of Thompson and Turnbull's cases, nor have

we seen it described elsewhere in the literature of Chiari's syndrome. We cannot explain its occurrence in our cases; there is no experimental evidence, for instance, that the toxins of senecio are excreted by this route.

Clinically, too, our cases presented as Chiari's syndrome, which is characterized by the rapid accumulation of ascitic fluid, hepatomegaly, abdominal pain, nausea, and vomiting. These signs and symptoms were present constantly, except that hepatomegaly was absent in one case and vomiting in 8. Splenomegaly is an inconstant finding in Chiari's syndrome and was not detected clinically in our cases.

From a study of the human disease alone it would appear that the primary action of senecio toxin is not on the parenchymal cells of the liver but on the central and sublobular veins. Elsewhere (Selzer, Parker, and Sapeika, 1951) the lesions produced by a senecio alkaloid in rats have been discussed and it was suggested that the primary effect on these animals is both on the liver parenchyma and on the central veins. In the latter an apparent proliferation of the endothelium of the central and sublobular veins, previously described by Davidson (1935), was found. Whether primary parenchymal damage and endothelial proliferation occur in man and are masked by subsequent changes, it is not possible to say. The endothelial proliferation could conceivably produce the lesion seen in man either by subsequent fibrosis or by superimposed thrombosis.

We have noted experimentally that a protein-deficient diet enhances markedly the effect of a senecio alkaloid. This may have a bearing on the etiology of the human disease as our cases all came from families in poor circumstances. Willmot and Robertson's cases (1920) had eaten a diet consisting of bread seldom supplemented by other food except sweet potato.

Species of senecio are known to be the cause of liver disease in horses and cattle—Dunsiekte and Molteno disease, respectively (Watt and Breyer-Brandwijk, 1932). Clinically, the similarity between the condition in animals and man is not striking. Dunsiekte is characterized mainly by abnormalities of behavior and other neurologic symptoms (Verney, 1911), while in Molteno disease diarrhea and abdominal pain are the most important features (Chase, 1904). Ascites is neither frequent nor marked (Theiler, 1919 and 1920), although the first of our patients stated that it had occurred in the animals in his district. On the other hand the resemblance of the pathologic features to the human disease is much closer. Theiler (1920) divided the histologic appearances in the liver of horses into early and late stages. The former was characterized by the presence of stasis in the central and

sublobular veins, extending into the adjoining capillaries. The vessel walls were said to be disrupted frequently so that pools of blood from which the liver cells had largely disappeared were formed around the central veins. The central and sublobular veins showed thickening of their walls, slight at first and marked at a later stage. The lumen was often eccentrically placed and sometimes several small channels were present. The early stage merged into a later stage in which the blood pools were no longer evident but in which there was marked fibrosis and bile duct proliferation.

We have been privileged to examine material supplied by Dr. G. de Kock from experiments performed by him on horses (de Kock, du Toit, and Steyn, 1931). In several of the sections the lesion described by Theiler in the central and sublobular veins was seen, and it corresponded very closely to the human lesion. It occurred less constantly, however, and it is possible that in horses primary parenchymal damage, the result of prolonged exposure to senecio, is of greater importance in the production of the marked fibrosis seen at a late stage of the disease. Again, there appears to be a species difference in the action of the toxin.

Finally, it remains to discuss the rôle of senecio in previously reported cases of Chiari's syndrome of unknown etiology. In the available literature—reviewed by Hoover (1920), Armstrong and Carnes (1944), Beattie and Hildebrand (1950)—we can find no cases corresponding closely to those described in this paper. In the great majority the primary vascular lesion was in the larger hepatic veins or inferior vena cava, and, although descriptions are inadequate to exclude an initial lesion in the smaller vessels, we consider that senecio poisoning has been responsible for few if any of the cases of Chiari's syndrome hitherto described.

Summary

This study included the clinical features of 12 cases of senecio poisoning with necropsy findings on the 6 patients who died.

Clinically, these cases presented as Chiari's syndrome, with a marked, rapidly developing ascites, hepatomegaly, and abdominal pain.

Pathologically, too, the features were those of Chiari's syndrome. The macroscopic appearance of the liver resembled that of chronic passive congestion and there was ascites and often marked edema of the large intestine. Microscopically, the liver cells in the centers of the lobules had disappeared and in their place were sheets of red blood cells. An occlusive lesion was demonstrated in the central and sublobular hepatic veins. Changes in the larger hepatic veins, when present, were considered to be secondary to those in the smaller vessels.

The human lesion is, in most respects, closely similar to the lesions of senecio poisoning in rats and horses. There appear to be some differences in the action of the toxins which are dependent upon the animal species concerned.

We wish to thank the staff of the Groote Schuur and Somerset Hospitals for permission to publish their cases; Professors M. van den Ende and F. Forman for much useful criticism; Mr. W. Taylor for the photographs; and Mr. E. Martin for preparing the histologic material. We are especially indebted to Professor J. G. Thomson for his help and for pointing out the resemblance of the lesion to that of Chiari's syndrome.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 135

FIG. 1. Case 2. Liver showing a marked nutmeg appearance, varying in degree from one part of the organ to another. A portal vein is occluded by a red thrombus. × 2/3.

FIG. 2. Case 7. Liver showing an occluded central vein. Foot and Day's reticulum stain. × 100.

FIG. 3. Case 2. Liver. A small hepatic vein is wholly occluded by tissue containing abundant argentophil fibers. Foot and Day's reticulum stain. × 100.

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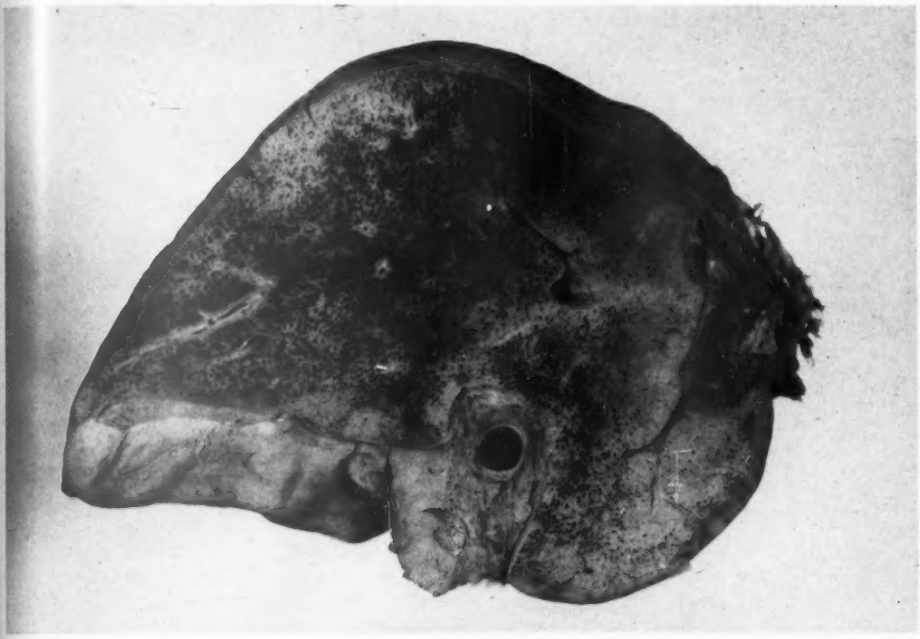

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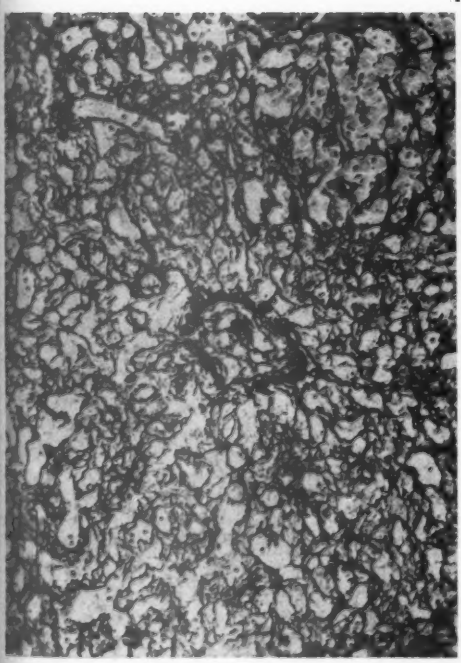

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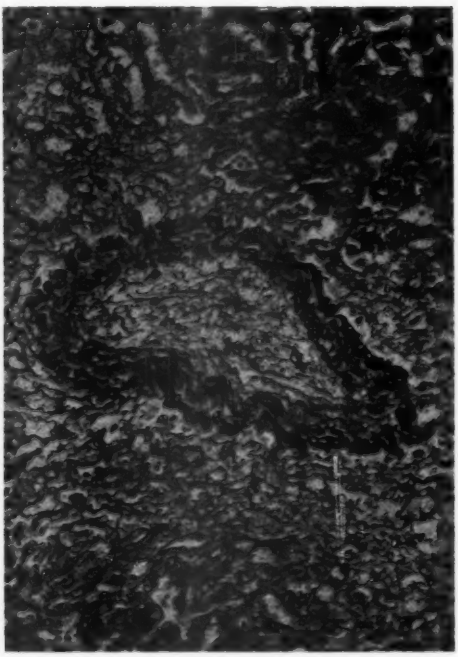

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Senecio Poisoning and Chiari's Syndrome

PLATE 136

FIG. 4. Case 7. Liver. Several hepatic veins are occluded by red thrombi. The liver is intensely congested. × 4/5.

FIG. 5. Case 4. Liver. Roughly one-half of the liver tissue is replaced by red blood cells. There is an increase in the portal connective tissue, slight bile duct proliferation, and lymphocytic infiltration of the portal tracts. Van Gieson's stain. × 100.

FIG. 6. Case 2. Liver. Two sublobular veins are shown, one wholly and the other partly occluded. Foot and Day's reticulum stain. × 100.

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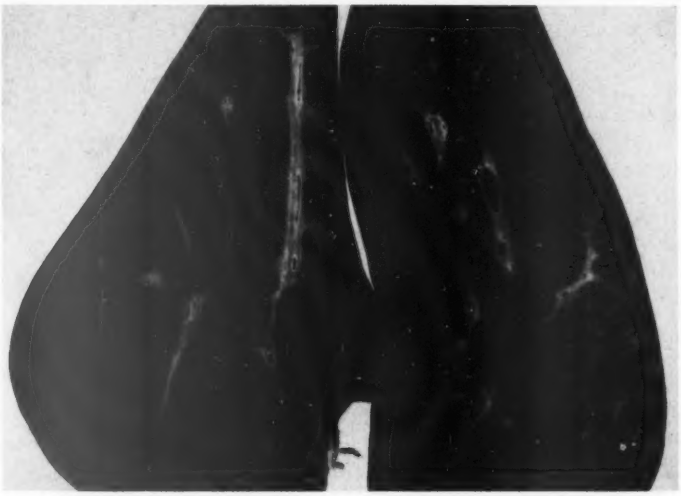

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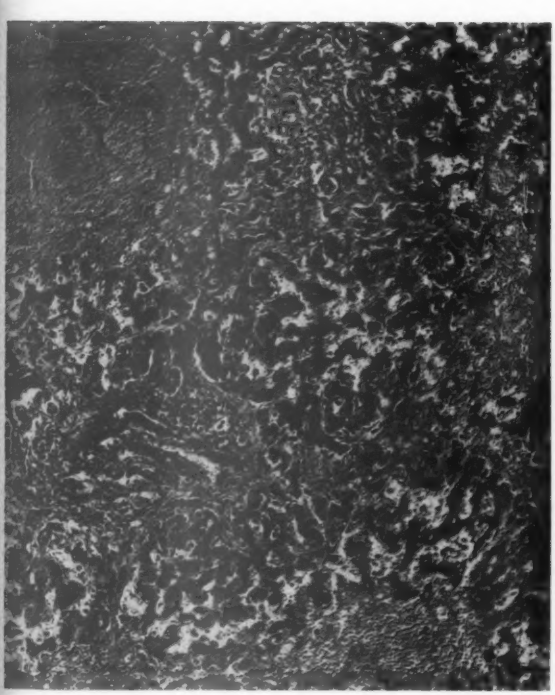

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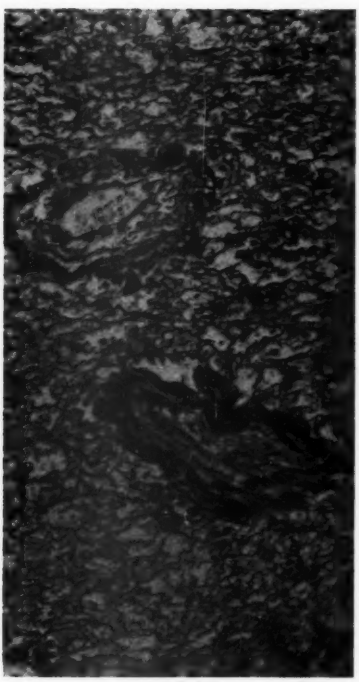

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Senecio Poisoning and Chiari's Syndrome

PLATE 137

FIG. 7. Case 7. Terminal ileum and ascending colon. There is marked mucosal edema of the ascending colon commencing at the ileocecal valve. × ½.

FIG. 8. Case 8. Liver showing a small hepatic vein with markedly reduced lumen. Foot and Day's reticulum stain. × 100.

FIG. 9. Case 9. Liver showing occlusion of a small hepatic vein. Foot and Day's reticulum stain. × 100.

WHAT'S NEXT?

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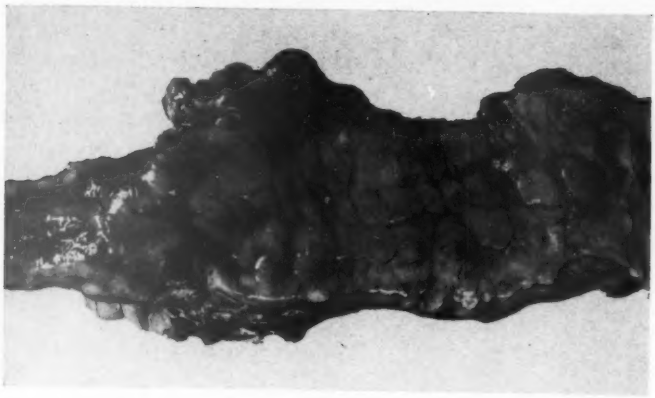

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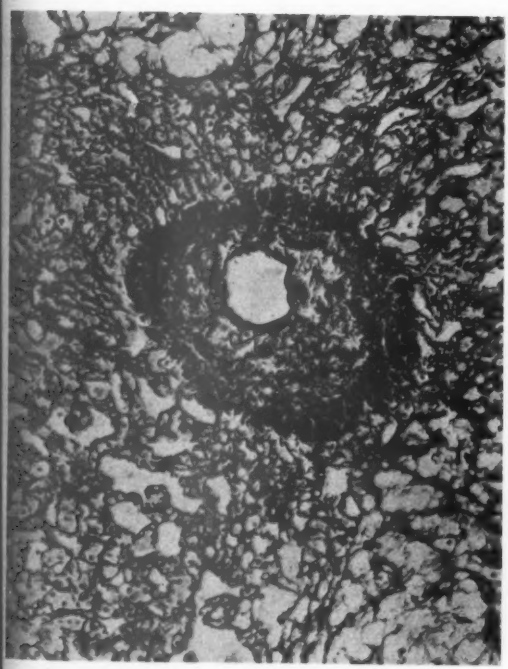

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Senecio Poisoning and Chiari's Syndrome

GRAPHITE PNEUMOCONIOSIS *

FREDERICK A. JAFFÉ, M.D.

(From the Department of Pathology, The Pathological Institute, McGill University, Montreal, Que.)

In the study of the pneumoconioses attention has been directed mainly to the effects of the inhalation of silica and coal dusts because both constitute major industrial hazards. Relatively little attention has been paid to the effects of graphite dust on the lung, in spite of the fact that large numbers of workers in many industries are constantly exposed to it.

Graphite or plumbago is one of the allotropic forms of crystalline carbon. It is black, oily to the touch, and has a metallic luster. Its most valuable physical properties are its oily quality, electric conductivity, resistance to high temperatures, and its ability to leave a mark on rough surfaces. Its industrial uses are thus many and include the manufacture of lubricants, polishes, electric batteries, electrodes, crucibles, furnaces, and lead pencils. It occurs in nature in crystalline or amorphous deposits and is obtained in mines or open quarries. The purity of the natural deposits varies within wide limits. Some deposits appear to consist of practically pure graphite, while others contain as much as 33 per cent of impurities, particularly silica and iron.[1] Very pure graphite can be produced artificially by the action of high temperatures on coke or anthracite. Persons engaged in mining or grinding graphite appear to be exposed to the greatest dust hazard.

Most of the investigations of the effect of graphite dust on the lungs of industrial workers have been based on radiologic evidence only. Kaestle [2] examined a large number of graphite workers and found evidence of pulmonary disease in only a few instances. He attributed the lesions in the lung to an admixture of silica in the inhaled dust. No increase in the incidence of tuberculosis was noted among these workers.

Dunner [3] and Dunner and Bagnall [4] reported the radiologic findings in 5 graphite workers. One of these had clinical and radiologic evidence of cavitation and expectorated large amounts of graphite. The necropsy findings in this case were later reported by Harding and Oliver.[5] While Dunner was at first inclined to attribute the pulmonary changes to the graphite itself, he subsequently reported a high silica content in the inhaled dust. Dassanayake [6] found radiologic evidence of pulmonary disease in a large proportion of graphite miners in Ceylon.

* Received for publication, January 4, 1951.

While these changes did not appear to have been characteristic of silicosis, he did not attribute them specifically to the inhaled graphite. Perry,[7] in a general review of the radiologic evidence, stated emphatically that "pure graphite will not produce fibrosis of the lung."

The first histologic study of the effects of graphite on the lung was that of Koopmann,[8] who described the necropsy findings in a graphite grinder who had died accidentally. He found a deep black pigmentation of both lungs and numerous small cavities in the bases of both upper lobes. Some of these cavities communicated with smaller bronchi. The main microscopic findings consisted of areas of diffuse fibrosis containing abundant, free, graphite crystals and occasional small collections of lymphocytes and plasma cells. The peribronchial and perivascular fibrous tissue was increased and contained many crystal-laden macrophages and occasional giant cells. Free crystals were found in the alveolar spaces and some of these crystals were said to be in the process of piercing the alveolar wall and entering the interstitial tissue. There was no evidence of tuberculosis. Graphite-containing macrophages were found in the peripheral blood, and small crystals in the portal spaces and Kupffer cells of the liver as well as in the glomeruli of the kidney. Graphite constituted 12.93 per cent of the dry weight of the lung tissue. No determinations of the silica content, however, were carried out, although the patient was stated to have been a stone grinder before becoming a graphite worker. Koopmann nevertheless expressed the view that the lesions in the lungs were to be attributed to the graphite dust.

The most recent and most extensive study was that of Harding and Oliver,[5] who reported the necropsy findings in 3 graphite workers. These had been engaged in mixing or grinding graphite for periods of 16, 19, and 23 years, respectively. All had complained of dyspnea and cough productive of black sputum for varying periods prior to death. The lungs were black, the pleura thickened and adherent to the chest wall. Two cases showed evidence of focal emphysema while in the third the emphysema was diffuse. All cases showed linear and radial fibrosis and 2 showed in addition hyaline nodules which were considered highly suggestive of silicosis. Numerous cavities in the upper and lower lobes of both lungs were found in the case which had been reported previously by Dunner and Bagnall.[4] Crystals of graphite were seen in phagocytic cells in the alveoli and in the areas of fibrosis. The lungs of all cases contained abundant refractile silica particles but none showed any evidence of tuberculosis. These workers attempted to reproduce the lesions in rats by the intratracheal insufflation of

graphite. Their animals developed a widespread granulomatous reaction with many foreign body giant cells. The authors concluded that the lesions found in their human material were primarily due to the silica component of the inhaled dust and that graphite pneumoconiosis should be considered an anthracosilicosis.

A similar view is expressed in the report of the International Labour Office on occupational diseases [1] which states that "it is not possible to speak of a specific risk due to graphite dust, any eventual risk being essentially dependent on the nature of the rock which constitutes the gangue."

In view of the great scarcity of histologic studies of graphite pneumoconiosis and the diversity of opinions regarding the nature and significance of its pathologic features, it is felt that this report of a single case is justified.

Case Report

The patient, a married man, 49 years old, was admitted to the Royal Victoria Hospital on February 5, 1950. During the past 8 years he had suffered from occasional morning cough which was productive of "heavy colored stuff." There had been some dyspnea on exertion during the past 5 years. The patient also gave an indefinite history of night sweats during the past 2 years. His appetite had always been good and there had been no loss of weight. Apart from the usual childhood diseases, there had been no illnesses or accidents. He was a non-smoker.

During a period of 5 years (1916 to 1921) the patient had worked in a graphite mine in Ontario. During that period he had been exposed to heavy dust. Since that time he had been the manager of a garage. He denied any other exposures to dust.

The patient's father had died of "lung disease" after having worked in the same graphite mine for many years. The nature of his illness was never established.

In November, 1949, the patient was examined in the course of a community chest x-ray drive. He was told that he had a tumor of the right lung, and he decided to come to the hospital for investigation.

Physical examination was negative. The tuberculin test was positive (1:1000) but gastric washings were negative for tubercle bacilli on smear and culture. Other laboratory investigations, including several cytologic examinations of the sputum, revealed no findings of significance. Roentgenograms of the chest showed a tumor mass in the region of the right upper lobe, either arising in, or displacing, the posterior lung field, lying immediately anterior to the fourth and fifth ribs and extending from the paramediastinal area well out to somewhat better than half the width of the thorax.

On February 16, 1950, a right thoracotomy was carried out (Dr. C. A. McIntosh). The operation report stated: "The entire lung was black and had a distinct metallic sheen. A tumour mass, the size and shape of an egg, was found in the postero-medial portion of the upper lobe. It was firm but not hard. There was no hilar adenopathy. A smaller nodule, 1 centimeter in diameter, separate from the main mass, was felt in the right upper lobe and a very small nodule in the lower lobe. A right upper lobectomy was performed. During the removal the main mass was ruptured and a small amount of watery, black material escaped."

The postoperative course was uneventful. There was excellent expansion of the remaining portions of the right lung and the patient was discharged on March 3, 1950.

PATHOLOGIC FINDINGS

Gross Examination

The specimen consisted of the upper lobe of the right lung measuring 15 by 12 by 5 cm. and weighing 195 gm. The pleural surface of the superior half was dull black and showed several areas of deep puckering, while that of the inferior half was smooth and dark red, with a distinct black mottling. A firm, irregular mass was palpable in the posteromedial portion of the lobe, immediately inferior to the apex. The remainder of the lobe was soft and doughy in consistency and appeared non-aerated. The apical branch of the eparterial bronchus was occluded 1 cm. from its origin.

Section of the lobe revealed an irregular, multiloculated cavity, which measured 5 cm. in its maximum diameter and was located in the center of the apical mass (Fig. 1). Its lumen contained a small quantity of inky fluid which on examination showed large numbers of graphite crystals as well as refractile, colorless, plate-like crystals. The latter were soluble in chloroform and gave a positive Liebermann reaction for cholesterol. The wall was black, ragged, and trabeculated. Several trabeculae were seen to bridge portions of the cavity. No communication between the lumen of the cavity and the bronchial tree was demonstrable.

The tissue surrounding the cavity for a distance of 1 to 2 cm. was dull black and very firm. It contained many small areas of necrosis, varying from 1 to 5 mm. in diameter, which were filled with a pasty, yellowish gray, cholesterol-rich material. This consolidated tissue was fairly sharply demarcated from the remainder of the lobe. The latter was dark red, atelectatic, and contained many firm, black nodules, 1 to 2 mm. in diameter, which became less frequent toward the base.

Two small, black lymph nodes were found in the hilum of the lobe.

Microscopic Examination

General Features. Sections of the lobe showed a granulomatous process characterized by the presence of graphite, large numbers of giant cells, areas of fibrosis and necrosis, and obliterative changes in blood vessels. This process involved all portions of the lobe but its extent and the morphologic details depended upon the amount of graphite present. In the basal portion the alveolar spaces were of normal size and contained clusters of mononuclear cells whose cytoplasm was filled with small, black granules. A few giant cells, with cytoplasm containing larger particles of a definite crystalline nature,

were found in the alveolar lumina. Occasional larger crystals, measuring up to 35 μ in their maximum diameters, were found free in the alveoli. The lining cells of the alveolar spaces were flat and devoid of graphite. The alveolar walls were delicate and contained occasional graphite-filled macrophages and giant cells.

The most pronounced change was seen in the adventitia of small arteries, particularly at their bifurcations. It was greatly thickened, often asymmetrically, and contained many giant cells and free crystals (Fig. 2). Many of the giant cells appeared to be located in perivascular lymphatics. Apart from this adventitial thickening, the walls of these vessels were normal.

A few fibrous nodules without blood vessels were demonstrable. Some of these adjoined a small artery but most were discrete and irregular, had scalloped edges, and occupied the area of 5 to 10 alveoli. Their fibrous strands showed a distinct tendency toward a radial arrangement (Fig. 4).

The air passages showed a slight deposition of graphite in their fibrous coats and collections of graphite-containing macrophages in their lumina. No nodule formation in relation to bronchioles was observed. The interlobular and subpleural tissues contained a few clusters of giant cells, but fibrosis was slight and patchy.

Sections from the middle portion of the lobe revealed considerably more graphite than those of the basal portion. The changes in the adventitia of arteries were similar but more widespread. Practically every artery was completely or partly surrounded by a dense black cuff consisting of graphite-laden giant cells and free crystals. Nearly all alveoli contained intracellular or free graphite. Many alveoli were lined by rows of giant cells which appeared to be attached to the alveolar wall. Others were completely filled by graphite-laden giant cells (Fig. 3). Occasionally one or two very large giant cells occupied the entire alveolar lumen. Close examination of those alveoli which contained many giant cells often revealed a delicate fibrous network obliterating the lumen and enmeshing the giant cells. Several dense fibrous nodules, essentially similar to those found in the basal portion, appeared to have arisen on the basis of this alveolar fibrosis. Their size and shape suggested that several alveoli had taken part in their formation. The fibrous tissue surrounding veins and air passages showed minimal graphite deposition and no striking increase in thickness.

Sections from the apex of the lobe showed the most graphite. In a few isolated areas the structure of the lung was still apparent. The

alveoli were collapsed or filled by giant cells, their walls were greatly thickened, and their lining cells were of a cuboidal type. In general, however, the normal architecture of the lung had been replaced by proliferating fibrous tissue and masses of graphite-filled giant cells. Many circular or oval areas of caseation necrosis, containing large cholesterol clefts and free graphite crystals, were scattered throughout this tissue. The lining of these necrotic areas consisted, in the majority, of cases of densely packed, graphite-containing giant cells (Fig. 6), while in a few instances it was formed by granulation tissue with numerous large cholesterol clefts, but little graphite. Thick wavy bands of hyalinized fibrous tissue were seen in all sections from the apical portion. These contained a number of large blood vessels and presumably represented thickened interlobular septa. Extensive patchy calcification was present throughout these bands. Two circular, laminated hyaline structures, reminiscent of silicotic nodules and measuring about 1 mm. in diameter, were encountered. One of these was enclosed in an interlobular septum while the other was in a granulomatous area but in contact with a band of hyaline fibrous tissue on one of its aspects.

All blood vessels showed adventitial thickening and well marked subintimal fibrosis. The latter was particularly conspicuous in the larger veins, many of which showed also large subintimal collections of lymphocytes (Fig. 5). Only in a few small arteries had the subintimal thickening progressed to the point of complete occlusion. The media of all vessels was relatively intact. Apart from the accumulations in the adventitia, no graphite was found in the blood vessel walls. No thrombosis was observed.

Many circular aggregations of lymphocytes, plasma cells, and occasional giant cells were scattered throughout the apical portion of the lobe, particularly in the vicinity of areas of necrosis and in the wall of the large cavity (Fig. 6). The lining epithelium of bronchioles was present in the center of many of these aggregations, its basement membrane resting directly upon the lymphoid stroma. In most cases the epithelium was complete and of ciliated columnar type. In a few instances it was incomplete and the cells showed a tendency toward squamous metaplasia.

Sections from the large cavity showed it to be essentially similar to the smaller areas of necrosis. Its lumen contained necrotic débris with cholesterol clefts and large amounts of free graphite. Its lining consisted of granulation tissue and masses of graphite-laden giant cells (Fig. 6). The trabeculae contained blood vessels which showed obliterative changes similar to those in the other blood vessels of the apical

region. The adventitia of these vessels also was greatly thickened and contained many giant cells and lymphocytes. The apical branch of the eparterial bronchus was completely occluded in the region of the cavity. The cartilaginous elements of its wall had been preserved but no trace of mucosa remained. The lumen was filled by fibrous tissue with many giant cells and free graphite (Fig. 7).

The hilar lymph nodes contained moderate quantities of free and intracellular graphite in their sinuses. The general architecture, however, was not disturbed and there was no fibrosis.

Giant Cells. Their high graphite content made the examination of the giant cells very difficult. A striking feature of those which contained little graphite was the presence of one or more asteroid bodies in the majority of such cells. These were of considerable size, often measuring 40 μ in diameter, and were frequently situated in close apposition to one another, thus giving the cytoplasm a foamy appearance (Fig. 8). These asteroid bodies never contained graphite and frequently had a distinctly hyaline appearance. They were more deeply eosinophilic than the remainder of the cytoplasm and their peripheral vacuoles were devoid of fat or glycogen.

Graphite. It had been hoped that measurements of the size of the graphite particles might throw some light on the pathogenesis of the lesions. Most of the larger particles, however, had undergone fragmentation during the process of sectioning so that no measurements *in situ* could be carried out. Some of the graphite was isolated by dissolving portions of the lung in concentrated sodium hydroxide. A black, crystalline powder was obtained which had a definite metallic luster. The softness of the crystals and their shape were characteristic of graphite. Microscopic examination revealed a large proportion of particles with maximum diameters of more than 30 μ, while those of a few particles exceeded 50 μ.

Silica. Visual examination under polarized light for the presence of silica was attempted. While a few, tiny, refractile crystals were found, the large amount of graphite made this method of examination unsatisfactory. The total silica content of tissue taken from the apex and from the base was then determined chemically. The following values were obtained:

	Tissue from the apex		Tissue from the base	
	Dry weight	Ash	Dry weight	Ash
	%	%	%	%
Ash	11.86		2.79	
Total silica	0.37	3.13	0.09	3.23

Tuberculosis. Sections from all blocks were stained for tubercle bacilli. Careful search, however, particularly in the areas of necrosis, revealed no acid-fast organisms.

Discussion

Owing to the nature of the case, this study must necessarily remain incomplete. It is apparent, however, that the inhalation of large quantities of graphite dust is capable of evoking a widespread, slowly evolving granulomatous process in the lung. This is characterized by nodular and diffuse fibrosis with areas of necrosis and cavitation, obliterative changes in blood vessels and bronchi, and large numbers of giant cells.

The clinical and pathologic findings lend support to the observations of previous investigators who found a slowly progressive process with marked predeliction for the upper portions of the lung, which tended to remain clinically silent for many years but which eventually might give rise to symptoms and disability. The absence of tuberculous infection is also in accord with the findings in previous cases.

The chemical findings indicate that the distribution of both graphite and silica coincided with the distribution of the lesions. Both the graphite content and the silica content of apical tissue were four times that of tissue from the base. The silica thus appears to have been introduced in admixture with the graphite. In view of the fact that some silica was present, the etiologic rôle of graphite cannot be established beyond doubt. The only 2 cases in which the silica content had been determined previously were those of Harding and Oliver,[5] and in these the total silica represented 1.14 and 1.77 per cent of the dry weight, respectively. The present case showed a mean total silica content of 0.23 per cent of dry weight which was considered to be well within normal limits. Apart from two vaguely suggestive nodules in the apical portion, the lung showed no morphologic evidence of silicosis. The conclusion, therefore, appears to be justified that the changes in the lung were due to the graphite itself and not to the small quantity of admixed silica.

The large size of the graphite particles indicated an inability on the part of the lung to eliminate particles of that magnitude. Their continued presence in the lung tissue evoked a granulomatous response which showed morphologic evidence of progression 29 years after the patient's last exposure to the dust. This effect of graphite stands in sharp contrast to the innocuous nature of inhaled amorphous carbon

and suggests that this difference in response is due to the difference in the physical state of the inhaled substance.

Graphite pneumoconiosis thus constitutes a form of "miner's lung" similar in many respects to that described by Gough [9] and Hepplestone [10] in Welsh coal workers. The 3 cases described by Harding and Oliver [5] appear to have shown a picture very similar to that found in the present study. The presence of innumerable giant cells throughout the lung does not appear to have been described in human cases. The closest approach to the lesions encountered in the present case appears to be that produced experimentally in rats by Harding and Oliver by the intratracheal injection of graphite. The presence of numerous asteroid bodies in the giant cells also has not been described previously and its significance remains unexplained.

Summary

In a case of pneumoconiosis due to the inhalation of graphite, the lung showed a granulomatous reaction, characterized by areas of fibrosis, necrosis, and cavitation, with obliterative changes in blood vessels and bronchi. Large numbers of giant cells were present throughout the lesions, many of which contained asteroid bodies.

The total silica content of the lung tissue was within normal limits. It is believed that the lesions in the lung are attributable to the graphite itself and not to admixed silica.

I would like to express my grateful appreciation to Dr. C. A. McIntosh of the Department of Surgery, The Royal Victoria Hospital, Montreal, for permission to report this case and to Professor T. R. Waugh of the Department of Pathology for access to the pathologic material. Dr. J. S. Stevenson of the Department of Geology, McGill University, rendered valuable assistance in the identification of the graphite. The determinations of the silica content were carried out by Dr. C. M. Jephcott of the Division of Hygiene of the Province of Ontario, through the courtesy of the Director, Dr. J. G. Cunningham.

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DESCRIPTION OF PLATES

PLATE 138

FIG. 1. Gross photograph of the superior half of the right upper lobe. An irregular, trabeculated cavity occupies a large part of the apical portion. The surrounding lung tissue is black and consolidated.

FIG. 2. A small artery shows an asymmetrically thickened adventitia which contains abundant graphite. A few giant cells are present in neighboring alveoli. Hemalum-phloxine-saffron stain. × 96.

FIG. 3. Low-power view showing patchy involvement of the lung. Some alveoli are lined by graphite-containing giant cells while others are filled by these cells. Small fibrous nodules are seen in the upper left and lower right. Hemalum-phloxine-saffron stain. × 23.

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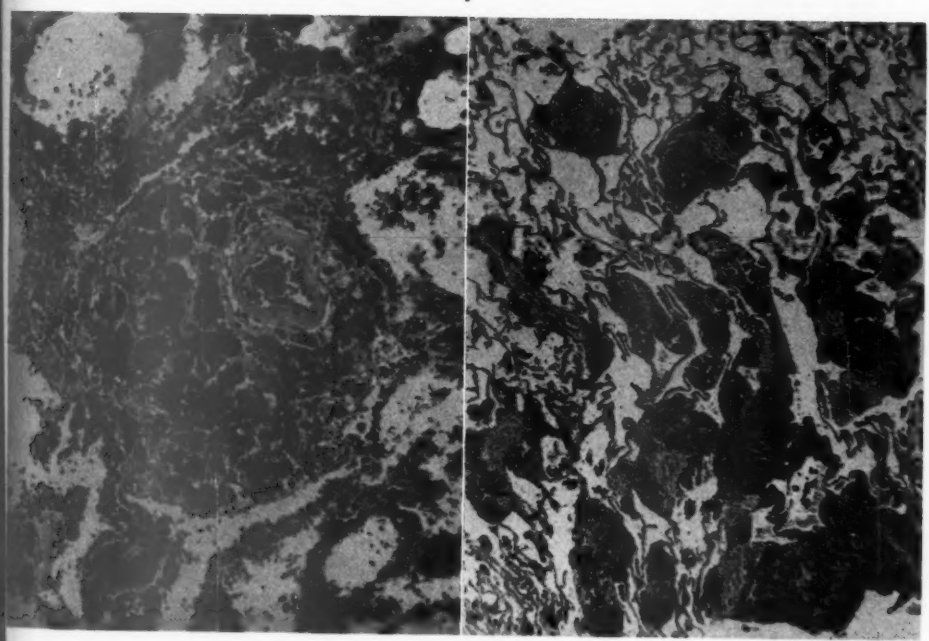

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Graphite Pneumoconiosis

PLATE 139

FIG. 4. Fibrous nodule in the lung containing abundant graphite and showing a radial arrangement of its fibers. It adjoins a small artery at its right inferior margin. Hemalum-phloxine-saffron stain. × 88.

FIG. 5. Large vein showing marked intimal thickening with infiltration of lymphocytes. The lumen of the vessel is seen at the upper left margin. Hemalum-phloxine-saffron and Weigert's stains. × 93.

FIG. 6. Section from the wall of the large cavity, the lumen of which is seen at the top. A small area of necrosis is present below. The intervening tissue contains several lymphoid follicles. Hemalum-phloxine-saffron stain. × 16.

FIG. 7. Cross section of the apical bronchus. Its mucosal lining has been replaced and its lumen occluded by fibrous tissue which contains innumerable graphite crystals and giant cells. Hemalum-phloxine-saffron stain. × 18.

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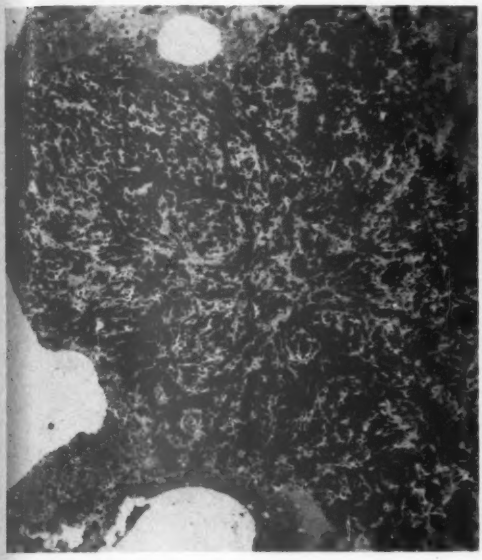

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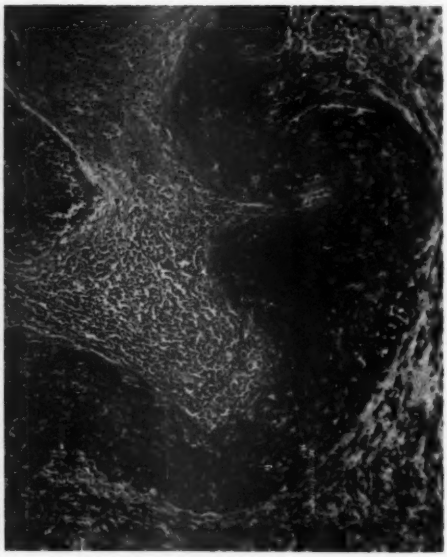

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Jaffé Graphite Pneumoconiosis

PLATE 140

FIG. 8. Large giant cell in a perivascular lymphatic space. The cell contains little graphite but there are three large asteroid bodies. Hemalum-phloxine-saffron stain. × 900.

FIG. 9. Photomicrograph of graphite crystals isolated from the lung tissue. The distance between the single vertical lines is 50 μ.

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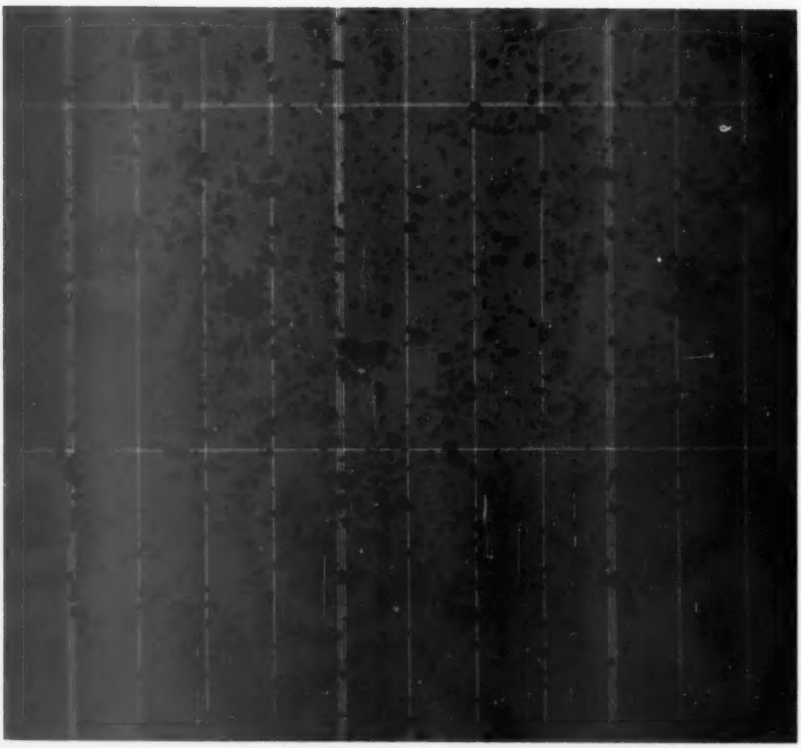

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